THE METHOD

OF

ENZYME ACTION

BY

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WITH INTRODUCTION BY

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TO M. B.

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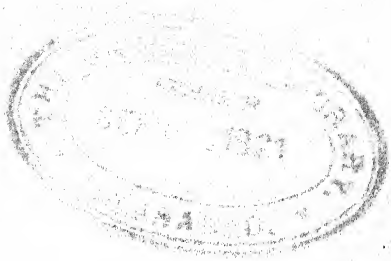


PREFACE

A THEORETICAL treatment of a scientific subject can lead only to a working hypothesis, and it is from this point of view that the theory of the mode of action of enzymes given at the end of this book should be judged. Owing to the circumstances in which the following pages were written, I have had neither the time nor the opportunity to verify the hypothesis by experiment. It is my hope, however, that the conception of enzyme action given will prove sufficiently interesting to induce some worker to test it and either prove or disprove it. From either result something of value will, I hope, be learnt.

The spinning of hypotheses without producing new facts to render them more plausible may seem a useless expenditure of energy, but it seems to me that if the subject has been carefully reviewed before the hypothesis is evolved in its final form—as I have tried to do in this book—at least two advantages are gained. In the first place the facts have been looked at from a new angle, and this may result in the observation of new relationships; and in the second place a working hypothesis may be reached, and this is the necessary prelude of fruitful experimental work. There is therefore, I think, a field open to anyone interested in a scientific subject who has not the capacity or opportunity to carry out experimental investigations, a field in which he can do useful work by

suggesting ideas which may lead a research worker to fresh lines of investigation.

In offering this working hypothesis for consideration I do not claim that the central idea is in any respect new. All I can say is that I have not met anywhere with the conception of the essential unity of the action of both hydrolysing and oxidising enzymes, nor an account of the mechanism similar to that given. It is quite possible, of course, that both have been previously suggested.

The books of reference used are as follows, the first three being those most frequently referred to and quoted from :

Bayliss, 'The Nature of Enzyme Action,' 3rd edition.

Do 'The Principles of General Physiology.'

Euler, 'General Chemistry of the Enzymes.' Translation by Pope.

Werner, 'New Ideas on Inorganic Chemistry.' Translation by Hedley (1911).

Kastle, 'The Oxidases and other Oxygen-catalysts Concerned in Biological Oxidations.'

Dakin, 'Oxidations and Reductions in the Animal Body.'

Mellor, 'Modern Inorganic Chemistry.'

Senter, 'Outlines of Physical Chemistry,' 4th edition.

Holleman, 'Textbook of Organic Chemistry.' Translation by Jameson.

Plimmer, 'Practical Organic and Bio-chemistry.'

Hatschek, 'An Introduction to the Physics and Chemistry of Colloids,' 2nd edition.

Willows and Hatschek, 'Surface Tension and Surface Energy.'

Wells, 'Chemical Pathology.'

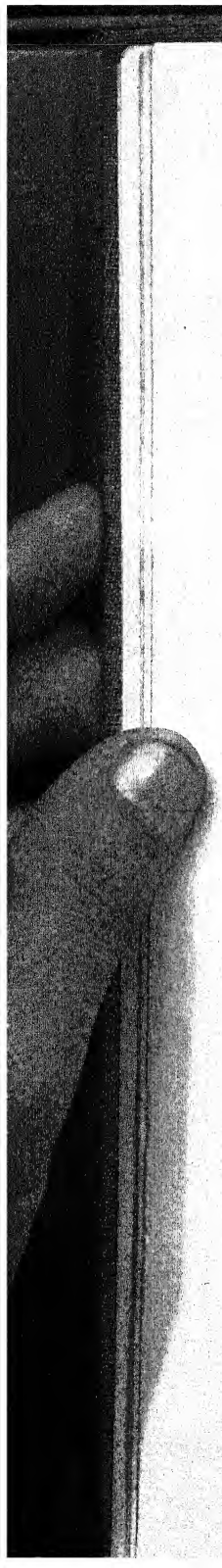
Moore, 'The Origin and Nature of Life.'

Access to original papers was impossible in the circum-

stances in which the book was written. The deep blue waters of the Mediterranean, now smooth as oil, now tossing the ship like a toy, the gorgeous sunsets, the brilliant stars of night, the glamour of Eastern ports, the unclouded sun, the sands seeming to quiver in the heat, and all the spiritual upheaval of this unprecedented time certainly stimulated imagination and effort, but made it impossible to quote from original sources.

My warmest thanks are due to Prof. (now Lieut.-Col.) Starling for his great kindness in reading the manuscript, for his advice as to publishing it, and above all for writing an introduction and thus multiplying many times the chances of the book being read.

March, 1917.



INTRODUCTION

BY

PROF. E. H. STARLING, M.D., Sc.D., F.R.S.

IN this monograph Captain Beatty deals with a subject far removed from the more primitive problems now engaging the activities of this half of the civilised world, though it is one that has attracted and will continue for many years to attract the interest of a large body of workers in science. Like so many others of the riddles with which life presents us, the mode of action of ferments fascinates both chemists and physiologists. In this riddle lies hidden the mechanism of most vital phenomena, and until it is solved we cannot hope to penetrate further into the workshop of the cell itself, or to understand the various intracellular activities which in their sum make up the life of the individual.

When Captain Beatty gave me his manuscript to read, written in the intervals of active service, I was struck with the lucid and concise manner in which he had brought together the chief results of modern research on enzyme and catalytic actions, and felt that his presentation ought to be made available to all workers in this field, as well as to the many others interested in the fundamental problems of life.

His survey, however, leads him to formulate—I think with more precision than has been done hitherto—a hypothesis of ferment action.

Life can be regarded as a development in the presence of the two essentials, air and water, probably under the influence of the energy of the sun's rays. Since the majority of vital processes are associated with some form of ferment action, which in turn is always connected with the mobilisation and shifting of OH and H ions, life itself might be regarded as the dynamical history of water.

The author tries to explain this mobilisation. He shows that for the directed reactions which are the distinguishing feature of living things and the condition of their evolution, two processes are required:

(*a*) Control of rate of reaction.

(*b*) Control of sphere of reaction.

These processes are achieved by the development of ferments, in the form of colloids or attached to colloid molecules, which are able to act only after adsorption, or attraction to the surface, of other colloidal molecules.

He shows that any enzyme displays two qualities:

(*a*) A power, common to all ferments, of attracting the H or OH groups in water.

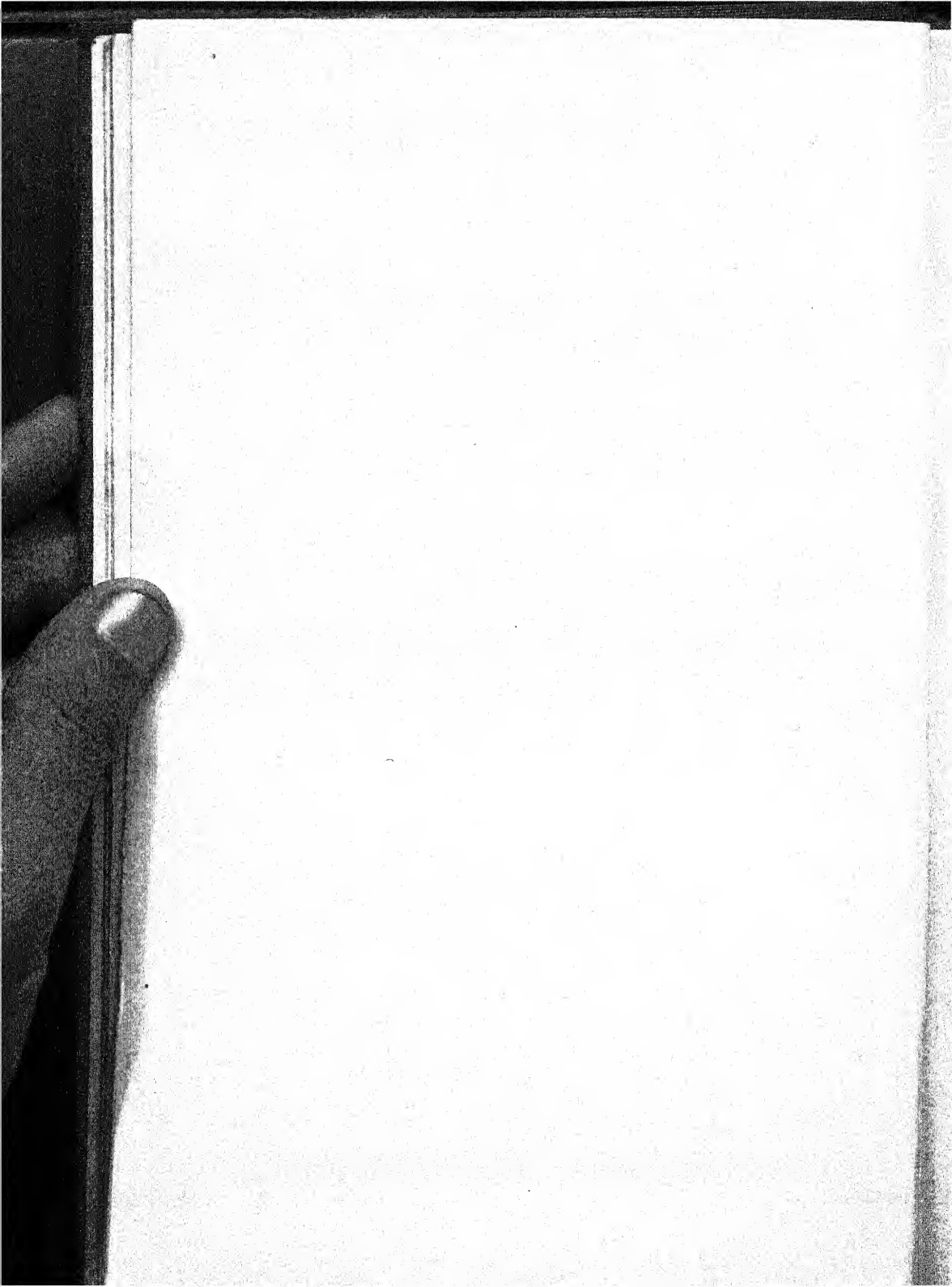
(*b*) A power, which is specific to the ferment in question, of adsorbing some particular substrate.

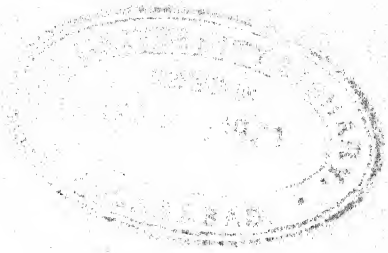
He suggests that these two properties, general and specific, find their explanation in a dual composition of the enzyme—which in every case must be compounded of one substance exercising the specific and another the general function. This is certainly true of some of the oxidases. These, however, according to the author, fall into the general category of ferments as mobilisers of H or OH ions. Their function is not as direct carriers of

oxygen, but as activating the H or OH group of the water molecule.

If the hydrolytic enzymes are built up in the same way the author points out that it should be possible actually to manufacture some of them, and boldly stakes the truth of his hypothesis on this possibility. The challenge is worth taking up.

SALONICA:
January, 1917.





CONTENTS

	PAGE
PREFACE	III
INTRODUCTION	VII
CATALYSIS	1
SUMMARY	7
GENERAL CONSIDERATIONS ON ENZYMES	7
COLLOIDS	9
SUMMARY	20
ADSORPTION	22
SUMMARY	32
THE PROPERTIES OF ENZYMES	34
SUMMARY	57
CHEMICAL ACTION. HYDROLYSIS	60
SUMMARY	81
CHEMICAL ACTION. OXIDATION AND THE OXIDASES	84
REDUCTION AND THE REDUCASES	99
SUMMARY	102
THE METHOD OF ENZYME ACTION. A HYPOTHESIS	105
SUMMARY	128
DEDUCTIONS	131
CONCLUSION	137
INDEX	139

THE METHOD OF ACTION OF ENZYMES.

THE numerous changes in chemical substances produced by the living organism can be imitated in the laboratory, but in many cases only by the help of powerful agents and with the application of a considerable amount of energy in the form of heat, electricity, etc. What is done with difficulty in this way outside the body is often done inside it with apparent ease, and with the help of trifling amounts of energy. The mechanism of these changes appears to be connected with the presence of enzymes, such as pepsin, trypsin, etc., and the nearest approach in ordinary chemistry to their action is that of catalysts.

Catalysis.

The characteristic action of a catalyst is to affect the rate of a reaction. Most catalysts increase this rate, but retardation is also known, as when the slow oxidation of phosphorus in air is stopped by a trace of ether vapour. Examples of the more usual type are: the power of finely divided platinum to bring about the combination of hydrogen and oxygen when the mixed gases are exposed to it; the influence of platinised asbestos on the oxidation of sulphur dioxide—if air and sulphur dioxide mixed

are passed through a porcelain tube heated to 400° C. then in the absence of platinised asbestos about 10 per cent. of sulphur trioxide is formed, but in its presence nearly 100 per cent.

While catalysts affect the rate of a reaction they do not affect the final result, and they themselves can be recovered unchanged at the end of the reaction. It is extraordinary what minute amounts of the catalyst can act. It is stated that one form of platinum can act on 1,000,000 times its weight of hydrogen peroxide, and the influence of molybdic acid on the decomposition of hydriodic acid by hydrogen peroxide is still greater.

A reaction which illustrates the qualities of a catalyst, and one which has been closely studied, is that of the evolution of oxygen from heated potassium chlorate when catalysed by manganese dioxide. Heat alone can cause the evolution of oxygen at a temperature of 350° C. After this has continued for a time the previously melted mass solidifies and now contains potassium perchlorate and potassium chloride. If the temperature is now raised over 600° C. the potassium perchlorate decomposes and more oxygen is given off; finally nothing is left but potassium chloride. The reaction follows a different course if manganese is first mixed with the potassium chlorate. Neither substance heated alone gives off oxygen at a low temperature, manganese dioxide requiring a temperature of at least 400° C. and potassium chlorate at least 340° C., but the mixture gives off oxygen at about 200° C.; when the reaction is complete potassium chloride alone is left except for the manganese dioxide, which can be entirely recovered. Other oxides can be used in place of manganese dioxide, though they do not act so vigorously, *e. g.* ferric, copper, cobalt, or nickel oxide. Now on close examination of the reaction it has been

found that the manganese is not quite unchanged at the close of the reaction; it is not changed in its amount or its chemical composition, but it is changed physically. Added in the crystalline form it has become amorphous. It appears also that it does enter into the reaction in the sense that the potassium chlorate does not give off its oxygen directly but instead oxidises the manganese dioxide, forming, it is believed, an unstable higher oxide of manganese. This higher oxide breaks up almost as soon as it is formed, liberating free oxygen and regenerating the dioxide. The regenerated dioxide is again oxidised and decomposed, and so the reaction proceeds to its end. Such a reaction, where intermediate products are produced which do not necessarily appear in the final products, is described as a "consecutive" reaction. Obviously at least two reactions occur, and it will depend on their relative speed whether the intermediate products can be detected or not. In the case where manganese dioxide was not used, potassium perchlorate was formed as a side reaction; but this cannot be detected when manganese dioxide is used, and probably none is formed. The manganese dioxide is said to catalyse or accelerate the main reaction, that of the evolution of oxygen, and in so doing it renders the high temperature at which potassium perchlorate would be produced unnecessary. A still closer study of the reaction shows that traces of other products are present, *e. g.* ozone, chlorine, and potassium permanganate, indeed reactions in general are rarely entirely confined to one or two products.

The formation of intermediate products, however, as an explanation of catalysis, is a hypothesis difficult to sustain when such catalysers as finely divided platinum, platinised asbestos, charcoal, etc., are in question, and if it is to be maintained an enlarged conception of the term

"intermediate product" must be formed. This meaning will be treated more fully when the conception of "adsorption" is considered, but meantime it may be pointed out that these substances appear to have the power of condensing certain gases and other substances on their particles. To such an extent is this possible that it has been estimated that gaseous carbon dioxide condenses on wood charcoal in layers about 0·005 mm. thick, and the gaseous layer is nearly as dense as liquid carbon dioxide. If two substances capable of inter-reaction are thus condensed together it must follow that their molecules have much greater opportunities of reacting than when not so condensed, and consequently the speed of the reaction must be enormously increased. Intermediate products in this sense are the physical unions of the reacting substances with the charcoal, etc., and the term "intermediate product" connotes not merely chemical but also what are called physical combinations.

Another objection which has been raised to the intermediate product explanation of catalysis is that in certain cases the obvious intermediate product which ought to be formed cannot replace the original catalyst in the reaction. For example, the catalytic action of hydrochloric acid in the formation of esters from methyl alcohol and acids is not shown by methyl chloride. This, however, merely proves that if an intermediate product is formed it is not methyl chloride. It will appear later that other intermediate products are possible when the solvent is taken into consideration.

While a catalyst can alter the speed of a reaction it cannot alter the final result. This is proved by a consideration of those cases where the products of the reaction remain in solution and are not removed from the sphere of action either by precipitation, as when silver nitrate

reacts with chlorides, or by evaporation, as when evolution of gas occurs. For example, when ethyl alcohol and acetic acid in molecular proportions react according to the equation:

$$C_2H_5OH + CH_3COOH = CH_3COOC_2H_5 + H_2O$$

it is found that when the reaction is complete the mixture has the composition 1/3 mol. alcohol, 1/3 mol. acid, 2/3 mol. ethyl acetate, and 2/3 mol. water, and these substances are described as being in equilibrium with each other. This reaction, when the pure substances are mixed with each other, takes weeks to reach completion, but in the presence of a mineral acid it is completed in a few hours. In spite of the difference of speed the final result, with or without the acid, is the same.

Again, it is found that the same result follows if instead of a mixture of ethyl alcohol and acetic acid, ethyl acetate and water are used in molecular proportions, and this occurs whether a mineral acid, such as hydrochloric acid, is used or not, though the speed of the reaction is enormously raised by the acid. This introduces the conception of "reversible" equations where the reaction can be started from either end; such an equation is usually indicated by the sign $\rightleftharpoons$ instead of the sign $=$. The first form of the equation may be considered as that representing the direct action, the second, the reverse action; and it follows from the direct experiments made that the catalyst accelerates the speed of both the direct and the reverse actions. Apart from direct experiment, however, this conclusion follows from the fact that the position of equilibrium is unchanged, for had the catalyst accelerated only the direct action while the speed of the reverse action—which must be coming into operation as soon as any molecules of ethyl acetate and water are formed—was unchanged the final result must have been an almost complete

transformation of the acid and alcohol into acetate and water. As this does not occur and the equilibrium is unchanged, the speed of the reverse reaction must have been accelerated equally with that of the direct.

Another result which follows from a consideration of these equilibrium equations is that where the products of any reaction are present in such a form as to be capable of reaction, reactions between them take place with resulting reformation of the original substances; in other words, the final position of equilibrium is due to a balance between two opposing reactions. In addition to this it is clearly possible for these products to alter the equilibrium which would occur in the absence of the catalyst if they enter into combination with it. Mere removal of the catalyst in this way from the operation would not affect the equilibrium, as the reaction slowed by this removal would still proceed to its normal end; but the position of equilibrium would be affected if the new substance produced by the combination of catalyst and product was capable itself of reacting with the bodies present in the mixture. In enzymes' action it will be found that there is evidence that these do combine with the products.

The amount of the catalyst added is of importance as regards the velocity of the reaction, for it is found usually that this is in direct proportion to the amount. At this point, again, enzymes are found to differ from inorganic catalysts.

Lastly, some catalysts are very specific in their action, failing to act in one of two closely allied reactions though acting on the other. As an instance, hydriodic acid can be oxidised with separation of iodine by hydrogen peroxide. This reaction is catalysed by tungstic acid, but the closely allied reactions of the oxidation of hydriodic acid by persulphates or bromic acid are not affected by tungstic acid.

SUMMARY.

(1) Catalysts are believed to be chemical substances.

(2) Their action is to affect the rate of a reaction.

(3) They do not affect the final result except in the rare cases where, perhaps owing to their complexity, new reacting bodies are formed with the products of the reaction.

(4) They exist unchanged in amount at the end of the reaction.

(5) They can act in very minute quantities.

(6) In many cases intermediate products are formed—sometimes chemical combinations, sometimes physical unions.

(7) The rate of the catalysed reaction varies with the amount of the catalyst.

(8) In a few cases their action is specific, reactions nearly related to that which they affect not being catalysed.

Enzymes. General Considerations.

The action of enzymes closely resembles that of the inorganic catalysts, but there are certain differences, depending apparently on their constitution and on their surroundings. In living organisms from the lowest to the highest they appear to be of universal occurrence, so much so that it is a question whether all the reactions of life do not depend on the presence of enzymes. It is true that there are many changes produced by living protoplasm which no enzyme as yet known is able to effect, but even these reactions may be carried out by protoplasm through the agency of enzymes having only a temporary existence. They are, however, plainly of simpler constitu-

tion than protoplasm, for the life of protoplasm is impossible in the presence of an amount of antiseptic which has little or no effect on enzymes. This may be due merely to the physical conditions of the cell, the antiseptic interfering with diffusion so that the exchange of cleavage products between different parts of the cell becomes impossible. Moreover, the distinction does not apply to all antiseptics, for certain enzymes are very sensitive to formaldehyde.

Provisionally, enzymes or ferments may be defined as the catalysts of living organisms. They may be divided according as they are usually excreted from cells into the surrounding medium, where they can be detected by their characteristic properties apart from the cells—ecto-enzymes—or they are contained only in the cells—endo-enzymes—from which they have to be extracted by special methods.

A distinction used to be drawn between "organised" ferments, such as yeast, and "unorganised," such as pepsin; but it has been shown by Buchner that by grinding up yeast cells in a mortar with sand and kieselguhr and then exposing the mass to a pressure of 300 atmospheres in a hydraulic press, a tissue juice can be extracted which, on purification from structural cellular elements, possesses the fermentative powers of yeast. It is believed, therefore, that yeast owes its powers to its content of unorganised ferments, and the distinction is no longer drawn.

Ectoenzymes may be obtained in an impure state by filtering the fluid in which the cells have been grown free from the cells, or in the case of glands by collecting the secretions; but the preparation of endoenzymes is more difficult, as the cells must first be ruptured before their contents can be obtained. This is accomplished by the following methods:

(1) Drying the cells at a low temperature, and sometimes warming subsequently to 50–60° C.

(2) Drying by short exposure to alcohol or acetone.

In both cases the material obtained is extracted with water; the aqueous solution is filtered and precipitated with alcohol.

(3) Permitting autolysis—self-digestion—to occur in the presence of toluene or other antiseptic. The enzymes pass into the solution.

(4) Mechanical disintegration, extraction with water, or by high pressure.

In both cases the material obtained is filtered and precipitated by alcohol.

Purification is difficult, and the methods used often affect the activity of the enzymes. The methods are:

(1) Precipitation and reprecipitation. Alcohol and acetone are generally used.

(2) Dialysis. In some cases co-enzymes are lost, or the enzyme passes through the membrane.

(3) Biological. This is possible in a few cases; *e. g.* in the case of amylase, which is not a food for yeast, the protein and carbohydrate in its solution can be used up by growing yeast in it.

In all cases the precipitate obtained can be dried only at a low temperature and at a reduced pressure.

Colloids.

Systems containing enzymes are heterogeneous—that is, they consist of more than one phase. The separate portions of matter of a system in equilibrium are termed phases, each phase being in itself homogeneous and separated by bounding surfaces from the other phases. This is implied in the statement that enzymes are colloids.

The character of colloids from which the name is derived is that they do not dialyse through parchment paper or other membranes. Examples are: solutions of gum arabic, albumin, and Bredig's colloidal metals formed by producing an electric arc between two terminals of the metal it is desired to bring into colloidal solution under water. Graham, who first studied the subject systematically, divided all substances into colloids and crystalloids, dividing them according to their capacity to pass through membranes or not. It is now known that a colloid, or rather the colloidal state, is only a *form* of matter, and that practically all substances can be brought into this state by appropriate methods. Even such a crystalloid as sodium chloride has been obtained in colloidal condition in petroleum ether.

Colloids are composed of one form of matter in a very finely divided condition distributed through a second, which may be a gas, a liquid, or a solid. The finely divided phase is called the dispersed or internal phase, the other containing it is called the continuous or external phase. An example of a mixture of gas in liquid is seen in foam, of liquid in gas in fog, of liquid in liquid in an emulsion (*e.g.* milk), of liquid in solid in jelly, of solid in gas in smoke, of solid in liquid in colloid suspensions, of solid in solid in some kinds of coloured glass.

The proof that these systems are really heterogeneous is furnished by an application of the Tyndall-Faraday phenomenon. A beam of sunlight or other powerful light, *e.g.* the electric arc, when passed through a darkened room makes visible the particles of dust floating in the air. This principle has been used in the ultramicroscope, where the rays from an electric arc are concentrated on a cell containing the liquid to be examined and viewed by a microscope placed at right angles to the path of the rays.

Two cones of light are seen, one converging to the focus of the rays, the other diverging. The fluid is strongly illuminated, and any particles in it are rendered visible. This visibility is due to the diffraction of light where it strikes each particle. The particle appears surrounded with a luminous zone, and the combination of particle and zone brings the whole within the range of microscopic visibility. There is a limit to microscopic vision conditioned by the wave-lengths of the rays forming the visible spectrum. These vary from 400 to 700 $\mu\mu$, and the limit of microscopic vision is reached at particles with diameters between 800 and 200 $\mu\mu$. The light diffracted by the particles is polarised owing to the small size of the particles (in a colloid solution), and this means that their diameters are less than the mean wave-length of the light forming the beam. Not all colloids examined showed separate particles; in many these are so fine that only a haze can be made out, but this is in itself proof of the existence of particles, for a perfectly pure, true solution is quite transparent. It must, of course, be predicated that the particles are either opaque or at any rate possess a different refractive index from that of the fluid containing them, or else particles, though present, would not be rendered visible.

The effect of this extremely minute subdivision of matter is to produce a great extension of the surface of the substance forming the disperse phase. An illustration will make this clear. A cubic centimetre in the form of a cube possesses a surface of 6 sq. cm.; if divided into 1,000 cubes the surface becomes 60 sq. cm.; if the subdivision is carried further till the edge of each cube measures only 10 $\mu\mu$, the aggregate surface is 60 sq. *metres*. The characteristic properties of colloids depend on their enormous surfaces. Specific surface is defined as the

surface which unit volume assumes through subdivision in any given system. Disperse systems begin to show their characteristic properties when the specific surface reaches the order of 10^5 sq. cm. Although the subdivision is so minute the particles are still very much larger than molecules, and that they differ essentially from molecules is indicated by the fact that the electric charge which they usually bear is variable both in sign and amount, while it is invariable in both respects in molecules when these possess a charge.

Colloid solutions are permanent; though composed of particles they do not gradually precipitate on standing. A colloid solution of gold prepared by Faraday fifty years ago is still preserved. There is more than one reason for this.

(1) It can be shown that the velocity of a small body falling in a liquid is, other things being equal, proportional to the square of the radius. Hence, if the particles are small and not much heavier than the liquid, a suspension will take a long time to show any marked clearing. But the fact that mere shaking up of irreversibly precipitated colloids will not reform the suspension indicates that other factors are at work.

(2) The electric charge on the particles. Most substances suspended in water have a negative charge. The origin of the charge is in doubt; it may be present naturally, or conferred on the particles by electrolytes. Surface ionisation, whether due to dissociation of the substance of the particle itself or to adsorption of outside ions, accounts for the majority, if not all the cases. From the point of view of the permanency of colloids, however, the existence of the charge is important, for it is clear that the particles, being all similarly charged, will repel each other, and the tendency to aggregation and precipitation is obviated.

(3) Probably the most powerfully acting cause at work is the constant motion of the particles. Even particles well within the range of microscopic vision exhibit constant motion, or what is known as Brownian movement from its discoverer. This is composed of an oscillating motion of particles round a central position, and an erratic translatory motion. The movements are seen in greatly intensified form in particles seen only by the ultra-microscope; indeed so active are the motions that they were at first thought to be something different from Brownian movement. The cause at root is probably the molecular movements which the kinetic theory of gases assumes, and indeed they furnish a direct proof of the theory. The constant bombardment which each particle receives from others must prevent its precipitation.

(4) The density of the medium; for the greater the density the less the effective weight of the particles.

(5) The viscosity of the medium. The greater the internal friction of the medium the longer will the particles take to fall under the influence of gravity.

In virtue of the large size, compared with molecules, of the particles which compose a colloid solution, colloids exert very little osmotic pressure, *i. e.* the pressure which a solute exerts on a solvent, and which is comparable to the pressure of a gas on its container, as is proved by the increase in the amount of solvent when put in a dialyser where the membrane is impervious to the solute but allows the solvent to pass. Diffusion to some extent, however, is possible in certain cases; for example, hæmoglobin diffuses slowly into gelatine, and some membranes are pervious even to protein if given time enough.

Colloids are divided into two main classes depending on the constitution of the disperse phase. Where this is a solid, the colloid has affinities with suspensions and is

described as a suspensoid; where it is liquid the affinity is with emulsions and the colloid is an emulsoid. The characteristics of suspensoids relate them to the macroscopic suspensions, but the emulsoids approximate much more closely to true solutions. This is indicated especially by their respective viscosities. The viscosity of a suspensoid is that of the continuous phase, while that of an emulsoid depends on the amount of the disperse phase and may be very great.

A closer examination of the emulsoids shows that the two phases present are similar in constitution, the continuous phase being a dilute solution while the disperse phase is a concentrated one. This is the case in those emulsoids where the same solvent is present in both. These are described as lyophile emulsoids. It may be asked why the two do not blend—why the phases remain separate. This appears to be due to the rigidity of particles of liquid when these are very small; thus the oil particles of a fine oil-water emulsion can be retained by some filters. Each liquid particle possesses so great an internal pressure preserving its shape that it cannot be distorted sufficiently to pass through the filter pores.

The two varieties differ greatly in their stability. Suspensoids are easily precipitated by electrolytes, emulsoids with much more difficulty. If to a suspensoid a trace of emulsoid is added, the suspensoid acquires in this respect the properties of an emulsoid, the cause appearing to be the deposit of a film of the emulsoid on each suspensoid particle.

Emulsoids are the more important class from the point of view of enzymes, as the colloids of the organism are lyophile emulsoids.

Emulsoids vary in their reaction to heat and in the influence of the amount of the solvent. There is the agar

and gelatine class which melt on heating and gelatinise on cooling, the liquid stage being termed a sol, and the solid a gel. The condition is reversible. Another case is seen in the only important inorganic emulsoid, silicic acid. This, when first formed, is in the sol condition, but on standing it is transformed into a gel, and the gel cannot be changed again into a sol. The change is irreversible, though no water separates. The course of the change is not like that of the solidification of molten metal, where the transformation is sudden and the course therefore discontinuous; it is continuous, the viscosity steadily increasing. Another form is illustrated by albumin, which does not form a gel on cooling or on increasing concentration, but which, when the temperature is raised to about 60° C., sets irreversibly. Previous to this denaturation occurs, so that the albumin comes to behave as a suspensoid and not as an emulsoid. Lastly, there are emulsoids like gum arabic solution, whose viscosity increases steadily on cooling or additional concentration, but which does not gelatinise nor does it set on heating.

The process of gelatinisation appears to be a change of distribution of the solvent between the two phases. It has been stated previously that the ordinary lyophile emulsoid colloid consists of a dilute continuous and a concentrated disperse phase. When gelatinisation occurs it is found that the continuous phase has become the more concentrated, and the disperse phase the more dilute. The sol of such a substance as gelatine therefore consists of a continuous phase of a dilute solution of gelatine containing liquid particles of a more concentrated solution. The gel, on the other hand, consists of a concentrated solution of gelatine as a continuous phase containing liquid particles of a dilute. Owing to the concentration of the gelatine in the continuous phase it has

some of the characteristics of a solid, and a gel is a solid containing liquid particles everywhere separated from each other by the solid phase. Under these conditions the liquid cannot be squeezed out without great pressure, and the condition is reversible. If gelatinisation is produced by formaldehyde the liquid phase is the continuous one, and the solid exists as a meshwork of threads. The first is an example of the vesicular type of gel, the second of the sponge-like. Obviously it is easy to squeeze the fluid out of the second form.

Electrolytes have a powerful action on colloids; in many cases precipitation or coagulation occurs. The suspensoid and the emulsoid colloids differ markedly in their sensitiveness, the suspensoids being easily precipitated, while the emulsoids require much stronger solutions of electrolytes to affect them. The difference, however, is only one of degree. Since suspensoids are kept in the permanent form largely by the electrical repulsion of their particles it is clear that any substance altering the charge must affect their stability. The charge can be affected by ions possessing a charge opposite to that of the particles of the colloid. As a rule, univalent ions, except hydrion, H, and hydroxidion, OH, added in the form of acid or alkali, have little effect, but the effects are marked if a divalent, and still more if a trivalent ion is added. The effect is apparently due to the alteration of the charge on some of the particles, so that they cease to repel and begin to attract those whose charge has not yet been altered, and as a result aggregation takes place, which, beyond a certain degree, becomes visible precipitation. If the precipitating power of a univalent ion be called x, then that of a divalent ion will be x^2, and that of a trivalent ion x^3. It is true that ions cannot be added separately; with a positive ion there must be added an

equivalent negative ion, but it is found that only the ion possessing a charge of opposite sign to that of the colloid particles acts—Hardy's rule. Emulsoid colloids, while largely insensitive to univalent ions, are readily acted on by trivalent ions. On precipitation the ions causing the coagulation are carried down in the precipitate; they can sometimes be removed by washing, sometimes directly, sometimes after prior treatment with a univalent ion, when the colloid solution is reformed, indicating that the ion has a physical, not a chemical, action on the colloid.

The effect of acids and alkalies is largely due to the power of their ions, H and OH, to alter the electric charge, for these ions, though univalent, have, as already stated, a powerful action, but they have other effects as well. It is well known that gelatine and other colloids can absorb great quantities of water, the process being known as imbibition. The amount of water which can thus be taken up is greatly increased by acid or alkali.

Imbibition is probably due to condensation of water on the particles of the colloid, leading to filling up of the capillary spaces between them, the particles themselves being unchanged in size. The forces developed are enormous; under a pressure of 41 atmospheres gelatine can still take up 16 per cent. of its volume of water, and at atmospheric pressure it can take up 330 per cent. (The swelling of a Laminaria tent is a familiar use to which this power is put.) It is found, besides, that though the gelatine has swollen, the bulk of the combination of water and gelatine is less than the bulk of the two taken separately; in the case of tragacanth the contraction is almost 2 per cent. This implies the compression of water, and to compress water to such an extent would require a pressure of about 400 atmospheres.

What applies to other emulsoid colloids applies also to the proteins, which are emulsoid colloids. In solutions which contain no electrolytes these are amphoterous, moving to either pole in an electric field, but any electrolyte, and especially acids and alkalies, confers a charge on the protein. As the amino-acids, whose combinations form proteins, are both acid and basic, this is due to combination of the electrolyte with the protein and subsequent ionisation of the combination. The absorption of water has been found to be increased greatly when this ionisation occurs, and this increase goes with other properties such as viscosity, solubility, osmotic pressure, coagulability by alcohol and heat, surface tension, and rotation of polarised light. Hence these effects of adding acid and alkali are really due to increase of water content, and this to the production of protein ions.

Neutral salts are in some respects similar to acids and alkalies in their action on emulsoid colloids. The effect is similar to that which occurs with suspensoid colloids, though less marked. The presence of an electric charge is obviously the cause, and therefore the effect of neutral salts is more marked when a charge has been conferred on the protein by the previous addition of acid or alkali.

Neutral salts have special actions on emulsoid colloids as well. Taking albumin and the effect, first, of cations, it is found that the salts of the alkalies produce coagulation or "salting out" only in great concentrations, and the process is reversible. The salts of the alkaline earths salt out in similar concentration, but the precipitate becomes insoluble on standing even for a short time. Finally, the salts of the heavy metals salt out irreversibly even in low concentrations. The anions, secondly, follow the order of the Hofmeister series. Beginning with the most powerful precipitant the series is: citrate, tartrate,

sulphate, acetate, chloride, nitrate, chlorate, while iodide and thiocyanate do not salt out even in saturated solution. The addition of thiocyanate to albumin can prevent its coagulation on heating even to 100° C. This order is followed when the reaction of the albumin is neutral; when it is faintly acid the order of the series is reversed. Now it is found that the salts of the Hofmeister series affect the properties of water such as viscosity, compressibility, etc., in the same order as in the salting out of albumin; it is clear, therefore, that the effect on albumin is due to the removal of water from the internal phase. When their water is removed the particles become solid and the emulsoid becomes a suspensoid, and so is easily precipitated by electrolytes, etc.

In addition to this effect of salting out, neutral salts combine with emulsoid colloids, but not apparently in the same way as do acids and alkalies, for while the latter frequently combine chemically as with the proteins, the combination with neutral salts appears to be of the nature of a physical union. They are, in fact, adsorbed. This accounts for the fact that the apparent solubility of comparatively insoluble salts is much greater in a solution of protein than in water. If, for example, phosphoric acid is added to a solution of casein in lime water, calcium phosphate is not precipitated, though it is formed.

It may be asked whether the heterogeneity of emulsoid colloids affects the chemical reactions taking place in them. It appears that in dilute solutions the effect is practically *nil*, the reactions taking place as rapidly as in water. In concentrated gels, however, diffusion is much slower than in water, and can be varied by the addition of various substances. The substances are those which affect the distribution of water between the phases, and it is hence believed that diffusion takes place in the liquid

phase. Again, reactions in gels do not proceed continuously, but the product when insoluble is deposited in strata separated by apparently clear intervals (Liesegang's phenomenon). This has not been satisfactorily explained.

The formation of colloids appears to be connected with complexity of constitution. Certain dyes like Congo-red possess such large molecules that their solutions in water take on the colloid form; in other cases increase in molecular weight is accompanied by a tendency to polymerisation and thus to pass into the colloid state. So the lower fatty acids ionise normally and show normal osmotic pressure; sodium oleate, on the other hand, in 20 per cent. solution has no osmotic effect, and therefore behaves as if it had infinitely large molecules. In more dilute solution it behaves more like a true solution.

Finally, colloid complexes can occur where the disperse phase consists of two or more constituents each of which is capable by itself of forming with the solvent a colloid solution.

Summary.

(1) Enzymes are divided into ecto- and endo-enzymes, of which the latter can be obtained only by rupture of the cells containing them.

(2) Enzymes are colloids. They do not diffuse through membranes.

(3) Colloids are heterogeneous systems consisting of matter in a finely divided condition—the disperse or internal phase—scattered through a homogeneous continuous or external phase. In many cases this can be proved directly by the ultramicroscope.

(4) The effect of the fine division of the disperse phase

is to extend its surface greatly. Many of the peculiarities of colloid action are due to surface action.

(5) Colloid solutions are stable, their stability depending partly on the electric charge of the particles, but mainly on their continual motion.

(6) Their osmotic pressure is very small, but in some cases measurable.

(7) Colloids are divided into suspensoids where the disperse phase consists of solid particles, and emulsoids where the particles are liquid.

(8) By their reaction to heat and other agents emulsoids can be divided into types, such as the gelatine, the silicic acid, the albumin, and the gum arabic types.

(9) The process of gelatinisation is due to the transference of the solvent from the continuous phase to the disperse phase.

(10) The action of electrolytes is in part due to their alteration of the electric charge on the particles. Acids and alkalies, in virtue of possessing H and OH ions, are very powerful in this direction. Acids and alkalies also increase the power of imbibition.

(11) Imbibition is a deposit of the solvent, water usually, on the particles, filling up the capillary spaces between them. The process is accompanied by compression of the water.

(12) Proteins are emulsoid colloids and show their properties. Acids and alkalies can combine with them chemically with later ionisation of the combination. This protein ionisation leads to increased imbibition.

(13) Neutral salts have several actions. They affect the electric oharge; they can extract water from the internal phase—salting out; they can unite with the particles by a physical union—adsorption.

(14) Reactions in dilute colloids are not affected, but

diffusion is slowed in concentrated gels. Reactions accompanied by precipitation show the precipitate in layers in the gel.

(15) Tendency to colloid formation is favoured by complexity of constitution, owing both to the size of the individual molecules and the tendency of complex molecules to polymerise.

(16) The disperse phase of a colloid may consist of more than one form of matter each capable by itself of forming the disperse phase of a colloid—a colloidal complex.

Adsorption.

There are certain compounds known which do not resemble chemical compounds in constancy of composition and more or less resistance to decomposition by physical agencies. These are the adsorption compounds. Examples are the compounds of charcoal and caramel, or charcoal and litmus formed when finely divided charcoal is shaken up with a solution of either of these colouring matters. The charcoal on settling carries the colouring matter with it, leaving the solution practically clear. Charcoal has also the power of taking up gases, especially those which are easily liquefied, such as ammonia or sulphur dioxide.

An examination of the conditions in which such compounds are formed shows that surfaces are always concerned, whether these are the obvious surfaces at the junction of liquids and gases, or the similar junction between a precipitate and a supernatant fluid, or the innumerable interfaces between colloid particles and the solvent in which they float. That many substances have a tendency to come to the surface is indicated by the

frothing which occurs when solutions containing soap, albumin, saponin, etc., are shaken. Each bubble represents an interface between the liquid and air, and if the whole be analysed it is found that the quantity of soap, saponin, or albumin in the froth is proportionately much greater than in the body of the solution. So condensed may albumin and saponin be that they form solid pellicles which are no longer soluble in the solution.

Adsorption compounds then are compounds formed at surfaces between the body possessing the surface and other substances which are in such a form, molecular or atomic, as not to possess a surface. Colloidal complexes have already been distinguished from these.

As is to be expected from the site of action, viz. surfaces, the quantity of any substance adsorbed is proportional to the active surface. Hence if the surfaces, say, of a colloid are already partly occupied by the same or another adsorbed material, less available or active surface is left for further adsorption.

It may be thought that the facts would be equally accounted for by supposing that there is a solution of the adsorbed body in the adsorbent, an absorption in fact, but the arguments against this seem conclusive. In the first place such a process would naturally be slow, as time would be required for the diffusion of the substance adsorbed into the adsorbent, whereas it is very rapid. Secondly, the ratio of the distribution of the substance between the disperse and continuous phase, if solid solution were the cause, would be independent of the concentration, provided the molecular weight of the solute was the same in both solvents. Applying this to a mixture of charcoal and water as representing the two phases, with acetic acid as the substance adsorbed it is found that the adsorption is not independent of the con-

centration, and hence either the molecular weight is not the same in the two solvents, or the explanation of a solution in the charcoal is incorrect. A calculation based on the assumption that the molecular weight is not the same shows that the molecular weight in the charcoal must be one-fourth only of that in water. Now acetic acid is known to exist in water as single molecules, hence it must exist in charcoal as quarter molecules, and this is impossible. The explanation of solution in the disperse phase is, therefore, incorrect.

It might also be thought that the compounds are chemical in their nature, but substances like argon can combine with charcoal which are incapable of entering into any reaction; also it is found that the composition of the compound of acetic acid and charcoal varies with the concentration of the acetic acid in the water, whereas a chemical compound should be constant in composition and independent of the concentration, at least over a certain range of concentration, assuming that more than one compound of the substances was possible.

It would, however, be a mistake to imagine that adsorption compounds are loose combinations which can be of any proportions in a given set of circumstances. In given circumstances of temperature and concentration these compounds are perfectly definite.

While in a case of partition between two solvents where a solute always distributes itself in proportion to its solubility in each, doubling the concentration doubles the concentration in both phases, and while again a chemical compound is not altered in amount by increasing the concentration provided the total amount of the reacting phase has been used up by the solute reacting with it, in the case of adsorption compounds increasing the concentration does increase the amount of adsorption com-

pound, but not in proportion to the amount of increase of concentration. Doubling the concentration does not multiply the amount of adsorption compound by 2, but by some root of 2, or, expressing it algebraically, by

$$\sqrt[n]{2}, \text{ or } 2^{\frac{1}{n}}.$$

Freundlich's general formula is:

$$\frac{x}{m} = aC^{\frac{1}{n}}.$$

In words: if x be the amount adsorbed by m grams of adsorbent, and C the final concentration after all adsorption has taken place, a and n being constants for a particular surface and solution, then the amount adsorbed by m grams of adsorbent is equal to a constant multiplied by the n^{th} root of the concentration. The law is not strictly accurate for extremes of concentration, for in these saturation of the adsorbent, if present in limited quantity, is possible, after which no increase in the concentration of the surrounding medium adds to the amount adsorbed, but it is in satisfactory agreement with the facts through a wide range of adsorption processes.

Since the amount adsorbed is related to the concentration, it follows that at a given concentration, the temperature remaining constant, an equilibrium is established between the adsorbed material and the surrounding solution, and that the equilibrium is completely reversible—diminishing the concentration diminishes the amount adsorbed. In actual fact, however, fixing of the adsorbed substance is often observed. This may be brought about by a physical change in the adsorbed material (which may become insoluble, for instance) or by a chemical reaction with the adsorbent, leading in many cases to the destruction or denaturation of the adsorbed material. This fixa-

tion is a relatively slow process and is non-reversible; it accounts for the fixing of various enzymes by charcoal, *e. g.* trypsin. In this special case the fixation is probably not due to a chemical reaction, for though water cannot remove the enzyme casein can.

It follows also from the law connecting adsorption with concentration that relatively more adsorption takes place in dilute solution.

An adsorbent is by no means indifferent in its action. Some substances are more strongly adsorbed than others. As will appear later, this is often due to the second reducing the surface tension more strongly than the first, and just as the components of chemical substances can be replaced by others, so in adsorption compounds one substance can replace another if it is more strongly adsorbed. The action of casein in breaking up the combination between enzyme and charcoal is an instance. Another is the effect of saponin on a charcoal-rennet combination. The action of rennet on milk is prevented by its adsorption to charcoal, but if saponin be added to such an inactive mixture the milk sets because the rennet has been displaced from its combination with the charcoal by the saponin.

The actual process of adsorption is rapid once the surface of the adsorbent has been reached. Cases which seem to show a time interval are probably really due to slowness of diffusion, not of adsorption, and consequently adsorption is hastened by shaking.

Heat has a double effect on adsorption. The actual rate of adsorption is increased, but the amount is diminished, for heat dissociates an adsorption compound. The explanation of this is found in the fact that surface tension has a negative temperature coefficient, *i. e.* it decreases with rise of temperature.

The condition of the adsorbed material from the point

of view of its reactivity is of importance. For the time being it adheres so strongly to the adsorbent that it cannot show its characteristic reactions. This applies to salts which are in the "masked" condition. Not merely can they not react chemically, but they cannot affect the osmotic pressure of the solution.

The causes of the formation of adsorption compounds seem to be mainly three—mechanical surface tension, the electrical conditions, and chemical attraction; but the surface film of any solution, including in this the films at the interfaces where there is a particulate phase, has properties which differentiate it from the remainder of the solution, *e.g.* potassium sulphate is 60 per cent. more soluble in the surface film. Also there are evidences of great powers of compression at the interface, as was mentioned in connection with carbon dioxide under the head of colloids. The tendency to adsorption of the easily liquefiable gases, sulphur dioxide and ammonia, comes under this head.

The action of the three main causes given is on the energy of the system, and is in accordance with the principle that the energy content of a system always tends to decrease. The Gibbs theorem states that if a body in solution by concentration on the surface of another phase lowers the surface energy, the process will tend to take place. Surface energy, however, is only one of the factors concerned; electrical, chemical, and thermal forces also play their parts. Hence Ostwald has generalised the theorem into a statement that increase of concentration at a surface will always occur when the potential of any form of energy at this surface can be diminished by the process.

That the surface of fluids behaves as if covered by a stretched film can be demonstrated by a loop of string in

a soap film. If the film be punctured through the loop, the loop will be drawn into a circular form, *i. e.* it surrounds as large an area as possible. The spherical shape of drops is another indication, as this shape possesses the smallest surface possible. This fact has been made the basis of measurement of surface tension by the stalagmometer. The cause of surface tension appears to be the unbalanced attraction of the molecules in the surface film by those at a greater depth from the surface.

Surface tension in fluids can be measured at the face between them and a gas or vapour, but the surface tension of solids in contact with a liquid cannot be measured directly; by indirect methods of measurement it appears to be much larger than the former; hence the extreme condensation which may occur or charcoal particles.

Most substances in solution lower the surface tension at a liquid-air interface; inorganic salts raise it, but not to any great extent. At the interface between liquids all substances, including inorganic salts, lower the surface tension. Since these bodies reduce the surface energy when at an interface, by the principle enunciated they must inevitably be forced to that interface, and hence adsorption takes place. Very great variations, however, are to be found in the power of bodies to lower surface tension; some, such as the bile salts, have a great effect; others, such as sugar, have very little.

The second force at work aiding adsorption is electrical. Electrolytes are adsorbed in much larger or smaller amounts than can be accounted for by the action of mechanical surface tension. Most substances in water have a negative charge, as mentioned under the head of "Colloids," therefore a positively charged body will be attracted to them. But if the adsorbent has a charge of

the same sign little adsorption will take place. It has been mentioned under the head of "Colloids" also that electrolytes can diminish, annul, or reverse the charge; if, therefore, a neutral salt be added the charge on the adsorbent will be altered and adsorption can take place. For the same reason adsorption is hindered by a neutral salt if the adsorbent and body adsorbed already have charges of opposite sign. With emulsoid colloids, however, their comparative insensitiveness to electrolytes tells against this reversal of the charge.

The relation of the electric charge to the surface tension is interesting, as its effect is to diminish the latter. Surface tension tends to pull the molecules of the surface film together; electric charge to force them apart. As applied to the disperse phase of a colloid the electrical forces are usually much stronger than mechanical surface tension. Lewis has shown that the amount of pure sodium oleate adsorbed by a water-oil interface is one hundred times greater than that calculated to be due to diminution of surface tension. Bayliss speaks of this as an example of the power of electrical adsorption, but Willows and Hatschek think that such substances as sodium oleate, methyl orange, Congo-red, etc., form colloidal and not true solutions, and that when they are adsorbed they form gelatinous or semi-solid skins on the adsorbing surface in which the concentration may be very great.

The third cause of adsorption is chemical attraction. While adsorption is generally the prelude to chemical action, and in the organism practically always precedes it, it does not follow that chemical action must follow adsorption. The two bodies attached may be incapable of reacting chemically, *e.g.* in the case of the adsorption of aniline to mercury. The attraction of an acid dissolved in the medium to a base forming, or on the disperse

phase of a colloid may be put down to this cause, or may be looked at as an electrical phenomenon. That the latter is the main cause is said to be proved in such cases as the union of Congo-red with thorium hydroxide, where a change of colour takes place on chemical union, but precipitation takes place *before* the change of colour. Nevertheless it is impossible to deny that chemical attraction may be at the root of such cases, for it is clear that chemical action cannot take place between two substances till their molecules come into contact, and it may be that in colloids the adsorption, which is a preliminary stage to chemical action, where this occurs, is caused by chemical attraction, and that the electrical charges have either only a secondary effect or are so intimately bound up with chemical attraction as to be inseparable from it.

There are a series of cases of adsorption which are not adequately accounted for by the effects of mechanical surface tension or by electrical attraction. These are spoken of as due to "specific" adsorption where a particular kind of surface takes up preferentially a particular substance. It appears that in these cases at any rate the chemical configuration of the surface must be taken into account. For example, carbon and red oxide of iron adsorb benzoic acid ten times as strongly as they do acetic acid, chromic acid adsorbs both acids equally, while platinum black adsorbs acetic acid slightly more strongly than benzoic acid, but neither to any very marked extent. Crystalline substances, such as barium carbonate, only adsorb crystalloids when these are isomorphous, *i. e.* crystallise in a similar form. Gelatine only adsorbs sugar after treatment with formaldehyde. The action of enzymes on optical isomers, an enzyme affecting one isomer and not, or only slightly, another, comes under this head. Indeed a great deal of enzyme action is bound up with

specific adsorption, and it is on this account unfortunate that the subject has not been more studied. Tentatively I suggest that chemical attraction lies at the root of these cases. Where chemical action is well marked it occurs so rapidly that the preceding adsorption is masked, or is practically simultaneous with it in colloid solutions. Where it is weak in the given conditions adsorption is separable from chemical action as in the case of Congo-red and thorium hydroxide, where the chemical reaction does not take place till the temperature is raised. The very different affinities of the elements for oxygen are cases in point. The gradations run from the intense reaction of metallic sodium or potassium in water to the practical absence of action with the "noble" metals. If a tendency to chemical action exists which may be fulfilled if the experimental conditions are altered, as in the case of charcoal in oxygen before heat is applied, then it is probable that an attraction which may be called specific exists between such bodies, and if one of these bodies possesses surfaces the phenomenon of specific adsorption will show itself. It is true that the examples given of specific adsorption do not look as if they were the preliminaries of chemical action, but Willows and Hatschek state that chemical combination cannot be rejected *à priori* even where it appears extremely improbable. Thus it is fairly definitely established that oxidation of carbon takes place when potassium permanganate is adsorbed by charcoal. The slow increase in the taking up of iodine by charcoal after the initial rapid adsorption may be due to a passage of iodine into the mass of the solid, but it is suggested by Freundlich that chemical combination is more probable, since iodine is a very reactive substance. The fact that the second part of the process is irreversible and does not vary with the kind of

charcoal used, while the first does and is reversible, is in favour of a slow chemical action.

Certain other facts bearing on the question of specific adsorption may be mentioned, though their explanation is still to seek. Gelatine takes up more acid fuchsin than Congo-red, while filter paper takes up the same amount of both. Gelatine takes up calcium salts more readily than potassium salts. If strips of filter paper are immersed in solutions of various salts it will be found that potassium salts rise higher than those of calcium and barium, though the water rises to the same height in all three cases. Mastic precipitates as adsorption compounds some of the proteoses in Witte's peptone, but not others. Kieselguhr takes from a mixture of the two proteolytic enzymes of the spleen large quantities of the *a*-protease, leaving the *b*-protease almost untouched. Possibly the electric charge of one protease differs from that of the other in sign. Invertase is adsorbed by certain inert powders but not by others. In the case of the proteases of the spleen charcoal adsorbs the same amount of both.

Summary.

(1) Adsorption compounds are formed at surfaces. The tendency of many substances to come to the surface is seen in froth.

(2) The quantity of substance adsorbed is proportional to the active surface.

(3) Solution of the adsorbed substance in the adsorbent does not account for the facts.

(4) There is an essential difference between adsorption and chemical compounds, especially indicated by the want of constancy of composition when the conditions are altered.

(5) In given conditions of concentration and temperature adsorption compounds are perfectly definite.

(6) The law relating the concentration of an adsorption compound to the concentration of the solvent shows that while the concentration increases with that of the solvent it does not do so in direct proportion.

(7) A reversible equilibrium exists between the adsorption compound and the surrounding solution. Fixation of the compound, however, sometimes occurs.

(8) Relatively more adsorption takes place in dilute solution.

(9) A substance which is more strongly adsorbed can replace one which is less strongly adsorbed.

(10) The actual process of adsorption is rapid.

(11) Increase of heat increases the rate of adsorption but diminishes the amount adsorbed.

(12) The adsorbed substance is so firmly bound to the adsorbent that it cannot give the chemical or physical reactions which it would give if not adsorbed.

(13) The causes of the formation of adsorption compounds are, mainly, mechanical surface tension, the electrical conditions, and chemical attraction, but changes in solubility and compressibility at the surface also play a part.

(14) These causes act by diminishing the energy of the system.

(15) The behaviour of a soap film and the shape of drops of water are proofs of the existence of surface tension.

(16) Electrical adsorption depends on the signs of the charges on the bodies affected, and these can be altered by electrolytes.

(17) Electric charges tend to diminish the effect of mechanical surface tension.

(18) Chemical attraction has probably a great influence, but this has not been fully studied. Specific adsorption may be due to chemical attraction.

The Properties of Enzymes.

Before discussing the method of action of enzymes it is necessary to understand clearly what their action is. The ground has been to some extent cleared by the discussion of colloids and the adsorption compounds which they form, and it will appear that enzyme action as compared with that of inorganic catalysts is largely modified by their peculiar physical form. Their resemblances to, and their differences from inorganic catalysts will require consideration.

(1) Unlike inorganic catalysts no enzyme has been isolated, so far as is known, in a pure condition, and their existence is recognised in a solution by their action. It is found that in reactions which can be brought about either by an enzyme or by an inorganic catalyst, the enzyme action is the more powerful of the two. Lactase has been found to hydrolyse about one-fourth of the milk-sugar in a 5 per cent. solution in one hour at 35° C., whereas even so strong an acid solution as twice normal hydrochloric acid required about five weeks to do the same. Another proof of the great activity of enzymes is furnished by invertase, which can hydrolyse 200,000 times its weight of cane-sugar.

The action of an enzyme, which alone furnishes the proof of its existence, can be investigated by discovering the chemical nature of the bodies that are formed, and when these are known by studying the rate of change and the various conditions which affect it. The chemical nature of the products is discovered by the usual chemical

methods, while for the rate of change various physical methods are available. These are mentioned in some detail by Bayliss. An enumeration of these methods may be of interest. The rate of change is related to the optical activity of the solution, its copper-reducing power, its refractive index, its viscosity, its molecular concentration as measured by its effect on the freezing point, and its electrical conductivity, the last being a method in frequent use. Where gas is evolved this can be measured, and the time during which it occurs. The spectro-photometer is used in colour changes, and the dilatometer in changes of volume. The difficulties of investigation are considerable in the later stages when the action is very slow, or when very small amounts of enzyme are present, for these are liable to destruction. The possibility that what is being measured in these cases is the slow non-enzymic rate of change has to be kept in mind.

(2) The action of enzymes is largely *specific;* they act on only one compound, *e. g.* invertase only on cane-sugar, or on only one group of compounds, *e. g.* maltase only on the α-glucosides, emulsin (from almonds) only on the β-glucosides. This differential action on optical isomers, which do not differ in chemical formula but only in the arrangement of one or more of their component radicles in space, so impressed Fischer that he compared the action of enzymes to that of a lock and key where only one key will open a particular lock, no other key will fit it, nor will its key fit other locks. This specificity is not a unique condition in chemistry. Euler points out that the reactivity of the simple and substituted mono-, di-, and tri-phenols is dependent on their constitution, whether oxidases or the inorganic manganese compounds as catalysts are used. For example, taking the dihydric phenols where in hydroquinone the OH radicles are in the para

position, in pyrocatechol in the ortho position, and in resorcinol in the meta position, the first is oxidised rapidly, the second more slowly, while the last is extremely resistant to oxidation. Again, lipase shows great differences in its power of hydrolysing such closely allied chemical individuals as ethyl acetate and butyrate, but hydrochloric acid also in relation to ethyl formate and methyl benzoate shows constants of hydrolysis in the ratio of 1·1 to 0·0003. The exact chemical constitution of the substrate, or substance on which the enzyme or inorganic catalyst acts, is therefore of great importance, though it may be that a similar chemical constitution is not incompatible with great physical differences. Even the specificity as regards optical isomers has chemical analogies, for if an optically active alcohol esterifies two opposed optically active acids it is found that the velocities of esterification are not equal. In general it may be said that two optical antipodes when combining with the same asymmetric substance do so with unequal velocities. An interesting consequence follows. Lipase is found to hydrolyse inactive menthyl mandelate, but forms the dextro component more rapidly than the lævo. This is exactly what occurs in the hydrolysis of the ester formed by an optically active alcohol with two opposed optically active acids, and hence it follows that lipase is optically active. Optical activity is widespread among the enzymes and appears to account for the great specificity shown in their action on optical isomers. Optically active catalysts of known composition act in the same way on optical isomers.

While a well-marked specificity is characteristic of enzyme action it would be a mistake to suppose that it is absolute. Lipase, for example, certainly affects the dextro-component of menthyl mandelate most, but it can

also affect the lævo-component. Taking the cases of maltase and emulsin as representing specific action in its most marked form, it is found that if these act together on glucose and alcohol, by synthetic action a mixture of the α- and β-glucosides is formed. If, however, they are added in sequence, so that the first has had time to have its full effect and therefore only one glucoside is present, the addition of the second, when time is given for its action, brings the position of equilibrium to the same position as if the two had been added simultaneously. In this case one glucoside must have been converted into the other, and the conversion has been brought about by an enzyme supposed to be incapable of acting on it. In fact, Fajans shows that the various experimental data as to the action of different enzymes acting on optical isomers, including synthetic action, can be explained by the hypothesis of both isomers being hydrolysed by the same enzyme but at different rates.

Nevertheless, when all deductions are made, the almost complete specificity of enzymes is an outstanding fact which has to be accounted for. The enzymes which hydrolyse the fats do not hydrolyse the proteins, and the same is true of the enzymes of carbohydrates. It is, however, difficult to admit the assumption that every substance found to be affected by an enzyme is affected by an enzyme which acts on it alone or on the small group of substances to which it belongs. Enzymes are found, like lactase in almonds, which have never had their specific substrate exposed to their action; if the assumption then were to be admitted, not merely would enzymes be numbered by hundreds, but it would be necessary to admit that enzymes are formed as by-products where they are never likely to be wanted. Probably the specificity is a group specificity, and any substance con-

forming in any of its parts to the physical or chemical type characteristic of the group can be affected by the group enzyme.

(3) It has already been pointed out in considering inorganic catalysts that in a reversible equation equilibrium can be reached from either side of the equation, and that if a catalyst catalyses the direct action it must also catalyse the reverse. As the changes produced by enzymes are generally of the reversible order, an equilibrium being produced, it follows that, given the appropriate conditions, the *reverse action* must be accelerated by the enzyme. Most enzymes have a hydrolytic action, one substance becoming two by the addition of the elements of water, hence the two substances can become one by the subtraction of water. The first definite proof of a synthetic action was brought forward by Croft Hill, who synthesised a form of maltose from glucose under the influence of maltase. Since then, "so many other cases have been discovered that the impression is distinctly given that it is merely a question of finding the proper conditions to be able to obtain synthesis from all enzymes" (Bayliss).

Most enzyme reactions are carried out in the presence of excess of water, and hence it follows that hydrolysis predominates over synthesis. In synthetic reactions the amount of water must be limited. A certain amount is necessary, for in the synthesis of a true fat from glycerine a certain small amount of water must be present, since if pure glycerine is used there is scarcely any synthesis.

There are many evidences of synthesis in the organism: the deposit of glycogen in the liver though glucose alone is brought to it, and the formation of proteins in the body though the proteins of the food are broken down to their amino-acid constituents by the erepsin of the intes-

tinal wall. Since water must be limited for synthesis to take place, special arrangements must exist for securing this, for there is excess of water in all the fluids of the body. Possibly there is actual solution in the disperse phase of the body colloids where water is nearly absent, or the protoplasmic sols everywhere present contain much less water generally than the body fluids.

E. F. Armstrong has expressed the view that enzymes do not build up the same molecules as those they break down, founding his opinion on the fact that Croft Hill's synthesis produced not maltose but isomaltose, and on other analogous facts. There is, however, a special explanation in Croft Hill's case given by Euler, and as enzyme preparations are as yet of no high degree of purity the usual view that the same molecules are synthesised as those broken down has much to recommend it.

Even when concentrated solutions are used, and the amount of water present severely limited, certain reactions proceed almost to the point of complete hydrolysis. The explanation probably is that the acceleration of the synthetic reaction may be very slow as compared with the hydrolytic. For chemical reasons there may be peculiar difficulties in the synthesis as in the case of the tertiary alcohol formed in the hydrolysis of salicin, which, like other tertiary alcohols, is esterified with difficulty.

(4) " The supposition that *combination* of the catalyst with the substrate yields the molecules which carry on the reaction has already received general acceptance in enzymology" (Euler).

" There is abundant evidence that a combination of some kind is formed between the enzyme and the substrate preparatory to the action of the former. There is also similar combination between enzyme and products " (Bayliss).

All the facts bearing on the specificity of enzymes confirm this conclusion, for these can only be explained on the hypothesis that enzymes form addition compounds with their substrates.

Again, enzymes are much more sensitive to heat when in a fairly pure condition than when either their substrates or products are present. For example, invertase will stand a temperature 25° higher in the presence of cane-sugar than in its absence, and it is also protected from the action of heat by its products. It is difficult to see how this could occur unless the enzyme entered into some kind of union with the sugar.

Another argument in favour of this combination is derived from the reaction velocity in the initial stages of certain enzyme reactions when low concentrations of the enzyme are employed. The rate of change is directly proportional to the time of action, *i. e.* equal amounts are changed in equal times. When the amount of substrate was varied, still keeping the enzyme low proportionally, in the early stages the amount inverted in equal times was nearly the same in all. The law of mass action was not followed. This can only be explained by presuming a compound between substrate and enzyme which persisted for an appreciable time, so that at first the enzyme was always in a saturated condition as regards the substrate and could not take any more up till that already taken up was hydrolysed.

Again, the enzyme may produce products which retard its action, precipitate, or even destroy it, this altogether apart from the slowing of the general reaction which necessarily occurs as the reverse action gathers way. For example, benzaldehyde is produced by emulsin and is deleterious to it. It is possible that these bodies influence the enzyme as a colloid and reduce the active surface.

These bodies could scarcely exert their influence without combining with the enzyme.

Besides the products proper the reaction of the medium is of great importance, and this may be altered inevitably by the course of the reaction.

The nature of the compound formed between the enzyme and its substrate is at first at any rate a surface condensation, an adsorption compound, for it is found that the law of adsorption is obeyed, and there is also definite evidence of the formation of such compounds. Amylase which can pass a porous clay filter by itself cannot do so when it is mixed with the calcium salt of caseinogen, on which it has no action whatever. Pancreatic lipase is entirely insoluble in fats, fatty acids, and solutions of fats in ether, yet it acts on these bodies, hence the reaction must take place in or on the enzyme, probably on it, for in the case of emulsin, which is able to hydrolyse its substrate in 90 per cent. alcohol (in which it is completely insoluble), there was in these circumstances no sign of imbibition. Again, a definite adsorption compound between starch and amylase has been observed. A 2 per cent. solution of starch remains unchanged alone, but if a small amount of amylase is added a precipitation gradually appears in half an hour or less. Hydrolysis takes place subsequently. Starkenstein has shown that adsorption may take place without chemical action in the case of dialysed liver amylase, but, generally speaking, adsorption is the preliminary stage of chemical action. Whether the enzyme enters into the chemical action or not will be discussed later when the method of action of enzymes is considered. Bayliss states that it is not known. He points out that mere concentration on the surface will by mass action greatly accelerate any process naturally occurring, but he does not think it possible

to explain all the various activities of enzymes in this way.

(5) In spite of certain differences in the course of their action the essential action of enzymes is *catalytic*. The resemblances to inorganic catalysts are much greater than their differences, especially if the fact is taken into consideration that the catalysis takes place in a heterogeneous system, not in a homogeneous. In a homogeneous system where all the reacting bodies are in solution it may be assumed that the reacting molecules are within each other's spheres of influence. In a heterogeneous system where one phase is particulate the facts, first, of diffusion to the particles by the dissolved substance, and, second, of its adsorption to the particles, must be taken into account, for these are the necessary conditions of chemical action. Both are rapid, nevertheless the rate of the reaction is necessarily to some extent conditioned by them. These considerations apply to catalysis in any heterogeneous system where the catalyst is in the particulate form, and, therefore, variations due to this seen in the action of enzymes cannot be put down to difference between enzymic and inorganic catalytic action.

The resemblances may be classified as follows:

(*a*) They increase the rate of a normally very slow reaction. Water alone at 100° C. can hydrolyse cane sugar to glucose at a measurable rate; it is probable, therefore, that the process takes place at room temperature at a very slow rate, and the action of invertase is to increase this rate.

(*b*) In certain cases they can be recovered at the end unchanged. It is here that, speaking generally, one of the differences between enzymes and inorganic catalysts is to be found. Nevertheless in certain cases the enzyme

can be extracted after the reaction is complete to hydrolyse fresh material.

(*c*) Minute quantities are capable of effecting a large amount of change. Facts bearing on this have already been mentioned.

(*d*) The curve of velocity of action resembles that of inorganic catalysts. At this point again differences are to be noticed.

(*e*) The enzyme has no effect on the final position of equilibrium. In certain cases a false equilibrium is reached owing to the destruction or inactivation of the enzyme before it has done its work. It is not a real equilibrium, for the normal uncatalysed reaction is still progressing, but so slowly as to be practically non-measurable.

Before classifying the differences it is desirable to point out on what factors the velocity of the enzymic reaction depends. It is clear that the velocity is determined by the concentration of the dissolved body in the colloid. Analysing this the factors are:

(i) The concentration of the substrate. The rate should be by mass action directly proportional to this concentration, but this only occurs when the concentration of the enzyme is not too low, else the enzyme will be saturated with the substrate without reducing appreciably the concentration of the substrate. There is also an upper limit to the concentration of the substrate beyond which action is again slowed. This may depend on the colloidal constitution of enzymes.

(ii) The concentration of the enzyme. If the substrate is in excess the velocity will be in direct proportion to the concentration of the enzyme, because the whole of it can enter into combination with the substrate. Later, when the substrate diminishes, the velocity will be a function of the

concentrations of both. At the end of the reaction, when the enzyme is in excess, it will again be a function of one only, but this time of the concentration of the substrate. With inorganic non-particulate catalysts it is usually found that the velocity of the reaction is in direct linear proportion to the amount of the catalyst added.

(iii) The presence or absence of electrolytes. As colloids enzymes are very sensitive to the action of electrolytes, but the effects produced will be discussed in a separate section.

(iv) The nature of the antiseptic added to prevent cell or bacterial action. Toluene is the most generally satisfactory as it is chemically inert. It is noteworthy that all enzymes do not react in the same way to the same antiseptic: to some it may be harmless, to others injurious. Obviously injury to the enzyme by removing the whole or part of it from the reaction interferes with the velocity of the reaction.

The differences between the actions of inorganic catalysts and enzymes may be thus classified:

(*a*) The velocity of reactions affected by inorganic catalysts depends on the concentration of the catalyst; of enzymic reactions at first on that of the enzyme, later on the concentration ratio between enzyme and substrate, and last on that of the substrate only, as discussed under (ii) above.

(*b*) The position of equilibrium is in some cases not the same under enzymic action and under the action of an inorganic catalyst catalysing the same reaction, *e.g.* an acid. The factors at work here may be:

(i) When intermediate compounds are formed their rates of formation and decomposition are not necessarily identical, so that in equilibrium there may be excess of one or other.

(ii) The heterogeneity of the system. For example, surface films have different powers of solubility from those of the body of the solution. It is found that the equilibrium of the hydrolysis of a salt of a fatty acid is altered by the presence in its solution of extensive surfaces.

(iii) A very small amount of energy is required to change the equilibrium point in reactions which are practically thermo-neutral. This energy may be derived from an obscure surface action.

(iv) If the enzyme takes up water the concentration of water at the point of action will be greater than in the body of the solution; this excess of concentration of water would mean a shifting of the equilibrium towards the point of greater hydrolysis.

(*c*) The products of the reaction directly influence the enzyme. The velocity of an enzymic reaction is altered by the addition of the products of the reaction to the system, not only as a condition of a reversible equation, as would occur with acid as a catalyst, but also by combination with and inactivation of the enzyme.

(*d*) Whereas great specificity of action is exceptional among inorganic catalysts it is the rule with enzymes.

(6) *Heat* has a marked effect on enzymes: most are destroyed by exposure to a temperature of 65-75° C., but there are exceptions. Some of the oxidases, *e. g.* medicago laccase, are not destroyed at 100° C., and trypsin in acid solution can be boiled. Since coagulable protein is practically always present the enzyme is precipitated probably because it is adsorbed to this, as occurs with fibrin ferment though this is not really a ferment. The effect of the presence of substrate or products is to diminish the sensibility to a raised temperature, as previously mentioned. As a rule enzymes are coagulated by heat or

precipitated, but in some cases the enzymes seem to be merely carried down by adsorption or changed in their physical state reversibly. As a general rule chemical reactions increase in speed by rise of temperature. Van't Hoff's rule is that for every rise of 10° C. the rate of a reaction is about doubled or trebled. This, the temperature coefficient, is high for enzymes, and it has hence been argued that the action of enzymes is chemical. But the temperature coefficient of imbibition is also high, so that the argument is of doubtful value.

If the action of heat on enzymes is similar to that of the heat coagulation of proteins the cause is probably the same, viz. a change in the state of solution. In many cases salts increase the stability of enzymes; thus the optimum temperature of pancreas amylase in a starch solution containing 0·2 per cent. of sodium chloride is at 50° C., while in pure aqueous solution it is at 35° C. The expression, optimum temperature, has no physical meaning beyond being a statement of the fact that "at a certain temperature the increased velocity of action due to this raised temperature is more than sufficient, for a time only, to counteract the rapid destruction of the enzyme" (Bayliss).

Extreme cold has no effect. Enzymes are not destroyed by exposure to the temperature of liquid air for two days.

At this point the effect of light and other radiations may be mentioned. The biological action of light on the inactivation of enzymes is of two kinds, one requiring the presence of oxygen and accelerated by fluorescent substances, the other produced only by the action of ultra-violet rays where no part is played by oxygen or by fluorescent substances.

Röntgen rays do not weaken enzymes.

Radium rays influence some enzymes, but not all in the same way.

(7) The action of *electrolytes* on enzyme reactions is marked. An ideal description of this action would divide the subject into (1) the action on enzymes themselves, and (2) the action on their substrates and products, and each of these would have two subdivisions according as the action was (*a*) chemical or (*b*) physical. It is not possible in the present state of knowledge to follow such an ideal treatment of the subject, as the exact method of action of an electrolyte is often unknown. On this account the subject will be divided according to the general action of electrolytes as adjuvants or paralysers, and a distinction will be drawn between acids and alkalies on the one hand and salts on the other.

A distinction must also be drawn at the outset between the more general action of electrolytes and the specific action of certain electrolytes on certain enzymes. These latter will be treated under the heading of "Co-enzymes."

As regards acids and alkalies it is found that while most enzymes are assisted by acids in low concentration others are inhibited and are assisted by alkalies, while others again act best in a neutral medium. Among the acid-assisted are pepsin, invertase, lactase, nuclease, and the autolytic enzymes. Those accelerated in action by alkalies are mainly the enzymes of the pancreas and intestine, and also the peroxidases. Malt diastase, blood lipase, and emulsin are best suited by a neutral medium. In all cases the concentration of the acid and alkali must be low: higher concentrations inhibit the enzyme action.

The action of acids and alkalies may be classified as follows:

(*a*) Conversion of proenzymes, or zymogens, into the active enzymes. This is especially well seen with pep-

sinogen, which is converted very rapidly by hydrochloric acid.

(*b*) They change the velocity of the reaction, which reaches a well-defined maximum for a certain concentration of the H ions.

(*c*) They influence the stability or decomposition of the enzyme itself, this stability exhibiting a maximum for a certain concentration of the H and OH ions, which is not necessarily the same as that for maximum action.

The sensitiveness of enzymes to acids and alkalies is very great, has been explained by the excessively small concentration of the enzyme itself. As to their method of action certain suggestions have been made.

(*a*) In many cases the concentration determining the optimum of acid or alkali must correspond exactly with the quantity of acid or alkali necessary for the neutralisation of the solution. This means that their action is on the H ion concentration.

(*b*) In some cases they act as activators of zymogens. Possibly the zymogen exists as a salt and the enzyme is set free by the acid or alkali.

(*c*) The acid or alkali may form a complex compound with the enzyme, which increases its activity, but this is pure hypothesis.

(*d*) In certain cases their action is on the substrate. In peptic digestion Euler believes that the hydrochlorides of the proteins are the molecules acted on, and in pancreatic the alkali salts. Analogies to this exist. In alkaline solution polyphenols are oxidised by the oxygen of the air with great readiness, but in acid solution only with difficulty. The action of lactic acid on the action of ricinus lipase seems due to this, as it has been shown that it has no action on the enzyme apart from the substrate.

(*e*) The acid or alkali may act as the common solvent, *i.e.* the common link between enzyme and substrate. This applies also to the action of salts. If instead of expressing the relation as that of a common solvent the action is considered as a facilitation of adsorption, Bayliss view of many cases is stated.

The action of salts hardly admits of classification.

Calcium and magnesium salts increase the adsorption of trypsin by paper (cellulose). As substances in aqueous solution generally have a negative charge, it is probable that this action of calcium is that of a divalent ion reversing the charge on one or other of the colloids, and so favouring adsorption. Calcium is said to be able to activate trypsinogen slowly, but its action can hardly be specific as it also accelerates the action of pancreas lipase. On the other hand, it has no influence on stomach lipase.

The action of ptyalin is diminished by the salts of weak acids. As these would hydrolyse to a small extent and the hydroxide of the metal, if an alkali metal, would dissociate practically completely while the weak acid would not, I suggest that the action on ptyalin is due to the occurrence of OH ions, for it is found that smal amounts of acid favour the action of ptyalin, while alkalies in similar concentration inhibit.

By dialysis, which removes electrolytes, certain enzymes can be rendered inactive, *e.g.* pancreas amylase. The addition of chlorides, nitrates, sulphates, phosphates, etc., of the alkali metals, but especially chlorides, restores the activity. So dialysed liver amylase is restored by sodium chloride. In a sense these salts are co-enzymes; but, on the other hand, they may be essential parts of the enzyme which is not an enzyme without them, but at best a zymogen. It has been stated that they enable adsorption

to take place, but adsorption can occur in their absence. Dialysed liver amylase is inactive; shaken with soluble starch at 40° C. and afterwards with rice starch in powder diffused through the solution, centrifuged, and filtered, the filtrate was found to contain no enzyme, for on addition of sodium chloride no sugar was produced. When the residue is similarly treated sugar is produced. I think it is clear that the residue must have adsorbed the enzyme, for, if not, a percentage of the latter would have been found in the filtrate, yet though adsorbed it is inactive without sodium chloride; therefore the action of the sodium chloride is on the chemical process and not on the adsorption.

Manganese is an essential constituent of most oxidases. Its action will be considered in connection with the oxidising enzymes.

Alkali phosphates accelerate the action of zymase and amylase, and they are absolutely necessary for the action of ptyalin and liver amylase.

Sodium chloride facilitates the action of amylase, maltase, and ptyalin, but retards the majority of enzymes.

Chlorates, nitrates, and hydrogen peroxide are deleterious to catalase, and hydrocyanic acid is especially so. The oxidising powers of the first three are clearly connected with this; the action of the last appears to be specific, as a strength of 1 in 1,000,000 reduces the velocity of reaction by one half (Euler).

In general, like acids and alkalies, salts must be used in low concentrations. In such concentrations possibly double decomposition occurs with alteration in the concentration of the H ions, these being apparently the real agents. In higher concentrations by their dissociation they hinder the dissociation of the protein salts, and it is possibly these which enter the reaction when dissociated.

Irreversible changes in the enzymes may also be caused by salts. The enzymes do not act apart from salts, and it appears to me that the active bodies formed by the combination, if not provided with their proper substrates, turn on themselves, so to say, and so the enzyme is gradually destroyed.

The special action of acid salts may be mentioned. These are well known to act as "buffers," *i. e.* they keep the H ion concentration steady, so that excess of acid, base, or salt, in the proportions likely to be found in life, can be absorbed so far as their influence on this concentration goes.

Non-electrolytes have little effect on enzymes, but the influence of narcotics may be mentioned. According to Euler the activity of those enzymes which are combined with the plasma in the living cell, *e. g.* zymase, is annulled by narcotics, while the enzymes occurring free in the cells, *e. g.* invertase, are not influenced. Meyerhof finds that the inhibitory effect of indifferent narcotics on enzymes is completely reversible, and interprets it as being due to the driving off of adsorbed substrate by the more strongly adsorbed narcotic.

In contact with collodion membranes most enzymes lose their activity and then exhibit a retarding action on fresh enzyme solutions. In seems to me that this might be accounted for on the same lines as for certain phenomena of dialysis, the collodion adsorbing some essential constituent of the enzyme, presumably a salt.

Some electrolytes have a poisonous or paralysing effect on enzymes. Some of these have been mentioned. Others are: perchloride of mercury is harmful to catalase especially, but also to amylase, urease, and trypsin, less so to erepsin and invertase; the strengths of the harmful solutions vary, but are always very small.

Cyanide of mercury has the same, but not so marked an effect. Calcium chloride weakens the effect of invertase, possibly owing to alteration of the electric charge. Iron salts are injurious to pepsin, and potassium permanganate strongly inhibits lipase.

(8) As described under the head of "Electrolytes" some enzymes require the presence of particular salts or other substances in order to manifest their action. Where one specific substance is required for a particular enzyme it is described as a *co-enzyme*. A distinction has been drawn between co-enzymes and the activators of zymogens on the ground that the action of co-enzymes is reversible —the co-enzyme can be extracted from the enzyme thus rendered inactive—while the relation of zymogen to enzyme is irreversible (Bayliss). Euler evidently thinks that the non-reversibility of the latter is not quite settled, as he says: "It is highly desirable that a more extended series of experiments should be made to decide if the conversion of pro-enzyme into enzyme is a reversible process." He considers that "there are no grounds for making an essential distinction between the activation of the zymogen with initiation of the reaction and the action of acids, alkalies, and many salts in accelerating the reaction." However this may be, it is usual to consider co-enzymes as reversible activators of enzymes. In some cases the co-enzyme is known; in others it is known to exist, but its composition is unknown. An example of the latter is found in connection with liver lipase. This is found in extract of liver. If the extract is dialysed it loses its power to hydrolyse lipoids. Add the dialysate and the power is regained. Boiled liver extract acts as well as the dialysate, as does liver extract from which the proteins have been precipitated by uranyl acetate. The activating body is precipitable by ether, but is soluble in alcohol; it

is not present in the ash of liver. It follows that it is thermostable, at any rate at the temperature of boiling water, that it is not a protein or adsorbed to proteins precipitated by uranyl acetate, and that it is decomposed on incineration. It is therefore not an ordinary mineral salt which would appear in the ash, but it might be an ammonium salt; the fact that it stands boiling is opposed to this. It might also be an organic body of comparatively simple constitution as it can be dialysed.

Another example is found in yeast juice obtained by Buchner's method. If this is filtered under pressure through a Martin's gelatine filter the colloids left behind are inactive, but mixed with the filtrate become active. This co-enzyme can be removed from the filtrate by dialysis and is not destroyed when the dialysate is boiled, but it disappears during fermentation or autolysis. Soluble inorganic phosphates greatly increase the activity of yeast juice, but they do not activate the inactive residue. It has, however, been proved that the presence of phosphates is necessary. Buchner has, therefore, inferred that the co-enzyme is an ester of phosphoric acid (Bayliss). Euler finds that lecithin and other organic compounds of phosphorus activate zymase, and states that these compounds constitute the active constituent in boiled pressed yeast juice.

An example of a known co-enzyme is furnished by bile-salts, as these are the co-enzyme of pancreas lipase. Sodium cholate is as active as the glycocholate, and the synthetic bile-salts have the same action as the natural bodies. It is not a question entirely of facilitating emulsion of insoluble fats, since the hydrolysis of esters which are soluble in water is also accelerated. The action is on the enzyme itself. Like other rapid activators, prolonged action causes destruction of the lipase. Bayliss suggests

that the action is physical and is due to increased subdivision of the internal phase of the lipase colloid producing a larger active surface, for bile-salts greatly lower surface tension. Other views are mentioned by Euler, viz. that the condition of solution of the substrate is influenced, or that a lipasogen is converted into lipase. It is to be noted that the lipase of the stomach and that of the intestines are not affected by bile-salts.

An interesting observation has been made connecting lipase and hæmolysis. Substances which exert a hæmolytic action, such as alcohol, soaps, saponin, digitoxin, increase the action of pancreas lipase, while this is annulled by cholesterol. Pancreas lipase itself becomes hæmolytic if activated by fat. As the hæmolytic substances can produce hæmolysis without the presence of lipase it is clear that there is some element common to cells, or rather to cell membranes, and to pancreatic lipase.

Other substances which have been described as co-enzymes are sodium chloride, which can activate dialysed liver amylase, and phosphates, which are absolutely necessary for ptyalin and greatly assist the action of zymase and amylase. These have been mentioned under the head of "Electrolytes."

Other substances which probably act in a different manner have been found in connection with the hydrolysis of starch. This is favoured (as well as by phosphates) by asparagine, amino-acids, proteins, and picric acid. An addition of 0·05 grm. of asparagine to 100 c.c. of a starch solution containing amylase increases the velocity sevenfold.

(9) Whether *anti-enzymes* can be obtained in the way that other antibodies can is a matter of dispute. Anti-emulsin was the first obtained, but Bayliss describes its

inhibitory action as entirely due to the change of H ion concentration, and if this change be imitated in a solution containing emulsin by the use of acid or alkaline phosphates the same inhibition is obtained. An antibody is obtained, he says, but only to the protein inevitably present in the impure solution of the enzyme. It is certainly remarkable that injection of an enzyme does not inevitably produce an antibody, *e. g.* none has been obtained for papain, and others are of very slight activity.

Another possible cause of anti-enzymic activity may be adsorption. The antitryptic activity of the serum has been found to be connected with the albumin fraction and not with the globulin as is usual with antibodies. Bayliss suggests that trypsin is adsorbed by the albumin and thus its action annulled. Serum albumin is difficult of digestion and is therefore little affected by the digestive action of the adsorbed enzyme.

On the other hand, Wells states that anti-enzymes obtained by injection are highly specific, *e. g.* the serum of an animal immunised against dog trypsin shows a much greater effect on dog trypsin than it does on trypsin derived from other animals.

The question, however, is not of great importance from the point of view of the method of action of enzymes. It arises chiefly in the discussion of the protein character or not of enzymes, as antibodies are apparently not formed against bodies other than proteins, with some doubtful exceptions.

It should be mentioned that some investigators have attributed a synthesising action to these anti-enzymes, but this is very doubtful.

(10) The existence of *zymogens* has already been frequently referred to. That the enzymes in the case of the secreting glands do not exist preformed but in a con-

dition from which the enzyme can be liberated at need may be a means of regulating the rate of enzyme action, also a means of defence of the body against its own enzymes, and, lastly, a method of storage.

The best-known zymogens are trypsinogen and pepsinogen. Trypsinogen is actually secreted as such, and only on meeting with the enterokinase of the intestine is the active trypsin formed. There is evidence that calcium salts also can activate trypsinogen, but the method of their action is disputed. It is suggested that traces of enterokinase are present in the juice as secreted, which, on precipitation of calcium salts already present, can act. That some other factor than calcium is necessary seems proved by the fact that calcium salts will not activate a juice which has been previously decalcified.

There is also a difference of opinion as to enterokinase. Bayliss and Starling think it an enzyme, stating that it can activate indefinite amounts of trypsinogen, others claim that a given quantity can activate only a certain amount of trypsinogen.

Pepsinogen can be obtained from the gastric mucous membrane after autolysis by special methods in a nearly pure condition. As the solution so obtained gives only two of the protein reactions, and those but faintly, pepsinogen must be of a non-protein nature. It is much less sensitive to alkali than pepsin, which is rapidly destroyed. Its relation to hydrochloric and other acids is of great interest, as nearly all the pepsinogen present in the aqueous extract of a cat's gastric mucous membrane may be converted into pepsin by 0·1 per cent. HCl in thirty seconds. As Glaessner remarks, the conversion by very dilute mineral acids with such smoothness, rapidity, and completeness is only explicable on the assumption of a very simple chemical process.

SUMMARY.

The properties of enzymes have been described under the following heads: 1. Recognition only by activity. 2. Specificity. 3. Synthetic or reverse action. 4. Combination with substrates and products. 5. As catalysts, with their resemblances to and differences from inorganic catalysts. 6. The effect of temperature. 7. The effects of electrolytes. 8. Co-enzymes. 9. Anti-enzymes. 10. Zymogens.

The following are the chief conclusions:

(1) No enzyme has yet been obtained in a pure condition, so that their existence is recognisable only by their activity.

(2) The methods of investigation of the activity of enzymes are: (*a*) chemical—the study of the nature of their products; (*b*) physical—the study of the rate of the reaction and the influences which act on it. Various methods are available for both.

(3) The action of enzymes is largely specific. This is especially seen in their relation to optical isomers. Analogies in general chemistry are to be found.

(4) Enzymes in general appear to be optically active bodies.

(5) The specificity, though great, is not absolute; it is rather a group-specificity than a substance-specificity.

(6) While the action of enzymes in aqueous solution is as a rule hydrolytic, with limitation of the amount of water the opposite reaction, that of synthesis of the products into the usual substrate, appears to be possible in all cases. Water must not be completely absent if the synthetic reaction is to succeed.

(7) This synthetic power of enzymes is of great import-

ance in the animal economy. Arrangements for the limitation of water are possible.

(8) The view that any enzyme can synthesise those molecules which it breaks down is not universally held, but seems the more probable view.

(9) "There is abundant evidence that a combination of some kind is formed between the enzyme and substrate preparatory to the action of the former."

(10) The enzyme also combines with the products of the reaction, and in some cases these retard, injure, or even destroy it.

(11) The compound formed between enzyme and substrate is, at first at any rate, an adsorption compound. Whether the enzyme enters into the subsequent chemical action or not is a matter for discussion; it is at the root of the whole question of the method of action of enzymes.

(12) The essential action of enzymes is catalytic. The differences between them and inorganic catalysts are partly due to the heterogeneous character of the system in which they act.

(13) The velocity of an enzyme reaction depends on (1) the concentration of the substrate, (2) the concentration of the enzyme, (3) the presence or absence of electrolytes, (4) the influence of the antiseptic used to exclude cell and bacterial action.

(14) The differences between the action of enzymes and that of inorganic catalysts are:

(*a*) The dependence of the action of the enzyme not merely on its own concentration, but also on the ratio of the concentrations of enzyme and substrate except at the beginning and the end of the reaction.

(*b*) The position of equilibrium is in many cases not the same when the same substances are cata-

lysed. The probable causes of this are enumerated.

(*c*) The products of the reaction influence the enzyme.

(*d*) Specificity is the rule with enzymes, the exception with inorganic catalysts.

(15) Most enzymes are destroyed by a temperature of 65–75° C. The effect of the presence of substrate or products is to diminish this sensibility to heat. Salts may also increase the stability of enzymes to heat.

(16) Light has considerable action, but this varies with the character of the light; the same applies to the effects of other radiations.

(17) The action of electrolytes on enzymes is marked. Acids and alkalies are especially powerful, but enzymes vary in their reaction to these.

(18) The action of acids and alkalies is shown (*a*) in the conversion of zymogens into enzymes, (*b*) on the velocity of the reaction, (*c*) on the stability of the enzyme. Various explanations are given, and it is especially necessary to keep in mind that the action may be on the substrate.

(19) Salts may act by changing the electric charges of the reacting bodies. This may occur through hydrolysis of the salt owing to increase of the H or OH ions.

(20) Some salts are absolutely necessary to the action of certain enzymes. It may be that they are essential parts of the complete enzymes.

(21) All electrolytes must be used in low concentrations when studying their effects; in higher concentrations all inhibit the action of enzymes.

(22) Narcotics may act through adsorption by the enzymes with displacement of the usual substrate.

(23) Some salts paralyse enzyme action, especially those of mercury.

(24) When a specific electrolyte (or non-electrolyte) is required by a particular enzyme before the latter can act, it is described as a co-enzyme. Bile-salts are the co-enzyme of pancreatic lipase, an organic phosphorus compound of zymase.

(25) Co-enzymes are reversible activators of their enzymes, and are thus distinguished from the activators of zymogens, but this view is not fully accepted.

(26) Besides electrolytes, protein, amino-acids, and other complex bodies can act as stimulators of certain enzyme actions.

(27) The question of the existence of anti-enzymes is in dispute. Their apparent effects have been put down to changes in the H ion concentration and to adsorption.

(28) Zymogens, or the precursors of enzymes, are probably of value to the body as a means of regulating enzyme action, and as a means of protection against enzyme action when the action is not wanted.

(29) The best-known zymogens are trypsinogen and pepsinogen. The activation of trypsinogen by enterokinase may be an enzyme action, but the view is disputed. The rapidity of the conversion of pepsinogen by hydrochloric acid indicates a very simple chemical reaction.

Chemical Action. Hydrolysis.

Euler gives a list of forty enzymes whose individuality has been established, but he states that there are many more, though in some cases, he thinks, their existence is not proved. At first sight, therefore, it would seem impossible to detect any chemical action common even to a large number, and certainly not to all. Nevertheless on examination it is found that, however complicated the constitution of enzymes may be, and however much they

may differ in composition, many of them act on their substrates in precisely the same way. Euler classifies them according as they are concerned with (1) hydrolysis, (2) decompositions, (3) syntheses, (4) transference of oxygen, (5) unknown processes accompanied by coagulation, (6) fermentations, (7) oxidations. More than half of the forty mentioned, however, act as hydrolysers, and it will be found on analysis of the action of some of the remainder that the action is really that of hydrolysis.

A typical hydrolysis is an interaction between a salt and water whereby free acid and free base, or an acid and a basic salt, are formed. Hydrolysis is thus a kind of reversion of the process of neutralisation of an acid by a base, or of a base by an acid. Except both acid and basic radicles of the salt are weak, hydrolysis is always small in amount, and in the case of a salt of a strong acid and a strong base, hydrolysis, except in special circumstances, does not take place, or only to an insignificant extent. In the case of magnesium chloride and water a certain amount of the magnesium chloride is decomposed and magnesium hydroxide and hydrochloric acid are formed.

$$MgCl_2 + 2HOH = Mg(OH)_2 + 2HCl$$

or possibly

$$MgCl_2 + HOH = Mg(OH)Cl + HCl.$$

Hydrolysis belongs to the class of reversible equations, and an equilibrium is established when the speed of hydrolysis is equal to the speed of recombination of the products of the hydrolysis. If one or both of the products of hydrolysis are removed from the sphere of the reaction, either by the formation of a precipitate or the liberation of a gas, the whole of the salt may be hydrolysed. If bismuth chloride, $BiCl_3$, be mixed with water, for example, the basic salt $BiCl(OH)_2$ is quantitatively precipitated, $BiCl_3 + 2HOH \rightleftarrows BiCl(OH)_2 + 2HCl$. In the case of

magnesium chloride simple solution of the salt in water is sufficient to produce hydrolysis, but in other cases solution in water, while sufficient to produce hydrolysis, does so only very slowly, and the action can be hastened by a catalyst. The hydrolysis of ethyl acetate is such a case. It has been mentioned already that under the action of HCl, acting as a catalyst, ethyl acetate takes up a molecule of water and is transformed into ethyl alcohol and acetic acid.

$$CH_3COOC_2H_5 + HOH \rightleftarrows CH_3COOH + C_2H_5OH.$$

Similarly enzymes acting as catalysts hydrolyse their substrates when water is in excess, and synthesise the products of such a reaction when it is in defect. In other words, the action of these enzymes is either to add water or to remove it.

Taking the enzymes which act on the three great classes of food-stuffs, the fats, the carbohydrates, and the proteins, it is found that their main action is to hydrolyse. Thus the lipases hydrolyse fats into fatty acids and glycerine.

$$C_3H_5(St)_3 + 3HOH = C_3H_5(OH)_3 + 3HSt$$

Glyceryl stearate or stearin — Glycerine — Stearic acid.

Under the head of the lipases may be classed the esterases, such as butyrase, which hydrolyse the esters of the lower fatty acids.

The enzymes of the carbohydrates may be typified by the inversion of cane sugar.

$$C_{12}H_{22}O_{11} + HOH = C_6H_{12}O_6 + C_6H_{12}O_6$$

Cane sugar dextro-rotatory. — Glucose dextro. — Fructose. lævo.

The lævo-rotation of fructose is stronger than the dextro-rotation of glucose, and so the mixture of the two is lævo-

rotatory. The same action (the taking up of water) appears in the hydrolysis of the glucosides.

The proteins and their derivatives are attacked in the same way. The proteins are the most complicated of the food-stuffs in chemical constitution, but at root they are mainly built up of amino-acids of which glycine or glycocoll is the simplest, $CH_2(NH_2)COOH$. If two molecules of this are conjoined as in glycylglycine, $CH_2(NH_2)CO.NH.CH_2COOH$, and this is hydrolysed, the equation is:

$$CH_2(NH_2)CO.NH.CH_2COOH + HOH = 2CH_2(NH_2)COOH.$$

As a matter of fact, boiling with one of the mineral acids splits up a protein into its constituent amino-acids. The albumoses, peptones, and polypeptides are simply stages on the path of the decomposition of the proteins, and their hydrolyses are of the same type.

Of simpler bodies subject to hydrolysis, urea may be mentioned. Under the influence of urease, ammonium carbonate is formed, which breaks up into ammonia, carbon dioxide, and water.

$$CO(NH_2)_2 + 2HOH = (NH_4)_2CO_3 = 2NH_3 + CO_2 + HOH.$$

The amino-acids themselves are attacked by the desamidases, ammonia being split off and the amino-acid converted into the hydroxy-acid. The type is:

$$CH_2(NH_2)COOH + HOH = CH_2(OH)COOH + NH_3.$$

A similar change takes place in the conversion of adenine into hypoxanthine, or guanine into xanthine, OH being substituted for NH_2, and H combining with NH_2 to form ammonia.

In general hydrolysis may be represented as the interaction of water and a substrate, with the result that one of the radicles of water combines with one part of the substrate and the other with the other:

$$XY + HOH = YOH + HX.$$

Of the other enzymes mentioned by Euler some, it is likely, are not really such. Fibrin ferment is not an enzyme. It is not destroyed by heat, and it enters quantitatively into the products of the reaction (Bayliss). Chymosin, or rennet, in clotting milk shows only a colloid, not an enzyme reaction. Indeed, Euler describes such actions as "unknown processes accompanied by coagulation."

Under the head of decompositions he places that of hydrogen peroxide into water and oxygen, and of hydroxynitriles into aldehyde and hydrocyanic acid. The first will be dealt with in connection with the oxidising enzymes. As regards the second, the action is usually represented as:

$$C_6H_5CH\cdot{}^{OH}_{CN} = C_6H_5CHO + HCN.$$

Rosenthaler considers the action of the enzyme as hydrolytic, but there is no evidence of this in the above equation, which shows simply a decomposition. If, however, an intermediate compound is formed with water, the hydrolytic action is plain:

$$C_6H_5CH\cdot{}^{OH}_{CN} + HOH = C_6H_5CH\cdot{}^{OH}_{OH} + HCN.$$

The intermediate compound shows two OH radicles bound to one carbon atom. Such a combination is exceedingly unstable, chloral hydrate, $CCl_3CH\cdot{}^{OH}_{OH}$, glyoxylic acid, $CH(OH)_2COOH$, mesoxalic acid, $HOOC.C(OH)_2.COOH$, being rare exceptions. That, however, it can be formed, even if but momentarily, is shown by the hydrolysis of ordinary nitriles.

$$CH_3CN + 3HOH = CH_3C\cdot{}^{OH}_{OH} + NH_3 = CH_3COOH + HOH + NH_3.$$

It is true that such compounds as the intermediate one

shown are not found, but their existence is by no means improbable, as compounds containing three alkoxyl groups exist, viz. the ortho esters, *e. g.*

$$CH_3.C.\begin{matrix}.OC_2H_5\\.OC_2H_5\\.OC_2H_5\end{matrix}.$$

The full equation then would be:

$$C_6H_5CH.\begin{matrix}OH\\CN\end{matrix} + HOH = C_6H_5CH.\begin{matrix}OH\\OH\end{matrix} + HCN$$
$$= C_6H_5CHO + HCN + HOH,$$

or an hydrolysis with subsequent loss of water by an unstable product.

Of the remaining classes, syntheses are with great probability considered to be merely the reverse action of enzymes. Processes involving oxidation or the transference of oxygen require separate treatment. There remains the class of fermentations—*e. g.* the transformation of sugar into alcohol and carbon dioxide.

Fermentation is not a simple process; glucose does not become alcohol and carbon dioxide by a single step. In the animal organism alcohol is not formed; but apart from this the steps of the process are probably the same as with yeast, the formation of alcohol taking place under the action of yeast owing to the absence of oxygen. The steps are believed to be, with the chemical changes required:

Glucose.

$$CH_2OH.CHOH.CHOH.CHOH.CHOH.CHO.$$

= Glyceric aldehyde, two molecules.

$$CH_2OH.CHOH.CHO.$$

This appears to be a case of hydrolysis taking place at the point underlined, thus:

$$- - CHOH.CHOH - CHO + HOH$$
$$= - - CH\begin{matrix}OH\\OH\end{matrix} + \begin{matrix}H\\H\end{matrix}COH - CHO$$
$$= - - CHO + CH_2OH - CHO + HOH,$$

with subsequent elimination of water from the aldehydrol, $- - CH\begin{matrix}OH\\OH\end{matrix}$.

= Pyruvic aldehyde. $CH_3.CO.CHO$.

This appears to be due to loss of water, with subsequent redistribution of the atoms in the molecule.

$$CH_2OH.CHOH.CHO = CH_2 : C(OH).CHO + HOH.$$
$$CH_2 : C(OH).CHO = CH_3.CO.CHO.$$

The change is exactly analogous to the formation of pyruvic acid from glyceric acid, on distillation with potassium hydrogen sulphate, by loss of one molecule of water.

Another analogy is found in the formation of acrolein from glycerine after loss of two molecules of water.

$$CH_2OH.CHOH.CH_2OH = CH_2 : C : CHOH + 2HOH$$
$$CH_2 : C : CHOH = CH_2 : CH.CHO.$$

= Lactic acid. $CH_3.CHOH.COOH$.

This is due to the hydration of the last, $CH_3.CO.CHO + HOH = CH_3.C(OH)_2.CHO = CH_3.CHOH.COOH$ with redistribution of the atoms.

= Pyruvic acid. $CH_3.CO.COOH$.

This involves oxidation and elimination of water.

$$CH_3.C\begin{matrix}H\\OH\end{matrix}.COOH + O = CH_3.C\begin{matrix}OH\\OH\end{matrix}.COOH$$
$$= CH_3.CO.COOH + HOH.$$

= Acetaldehyde. $CH_3.CHO$.

This appears to be accounted for by hydrolysis with formation of carbonic acid, which breaks up into carbon dioxide and water. The hydrolysis takes place between the CO and COOH radicles.

$$CH_3.CO.COOH + HOH = CH_3.C\overset{H}{O} + HO.COOH$$

$$CO\overset{.OH}{.OH} = CO_2 + HOH$$

= Carbon dioxide and water.

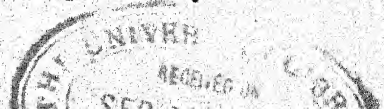
This involves oxidation.

$$CH_3.CHO + 5O = 2CO_2 + 2HOH.$$

Whether the explanations given of the actions represent the facts accurately or not, they show at any rate that the whole complex process can be represented as a mixture of hydration, dehydration, hydrolysis, rearrangement of atoms, and oxidation, and that these processes are the only ones actually required, even though the changes which really occur may differ to some extent from the picture given.

All the classes so far examined, therefore, omitting the coagulation processes as being colloidal, not chemical reactions, are connected with hydrolysis through hydration and its opposite. In order, therefore, to understand enzyme action it is necessary to form as clear a conception as possible of what occurs during hydrolysis apart from catalytic action, such as happens, for example, when magnesium chloride is dissolved in water.

It must first be premised that when a salt like potassium chloride, KCl, is dissolved in water it exists in the solution partly as molecules of KCl and partly as charged atoms of K and Cl, the metallic radicle bearing a positive charge and the acid residue a negative. These charged atoms are known as ions; they may be atomic, as in the case given, or compound radicles like NO_3, SO_4, etc. The oppositely charged ions are not completely free from each other; there is an electrostatic attraction between them which prevents their complete separation by mechanical means.



Some of the facts which have led to this conclusion may be briefly stated as follows.

The osmotic pressure of a solution of, for example, sugar conforms to the laws which express the action of a gas in a closed container, for in the case of a solution the container is the solvent and the gas is represented by the solute. It has, however, been found that the osmotic pressure for bases, acids, and salts, calculated on the assumption that they are present in solution as molecules, does not agree with the observed facts, but that the pressure is higher than it should be; in fact the solution behaves as if there are more active particles present than there are molecules.

It was next shown by Arrhenius that only those solutions which have an abnormally high osmotic pressure conduct the electric current.

Faraday had previously shown that when an electric current is passed through such a solution chemical changes occur like the development of acid round the positive pole and alkali round the negative. It was clear, therefore, that matter travelled with the current, and to these travelling parts of the solution Faraday gave the name of *ions*; those going to the anode or positive pole were called anions, and those to the negative or cathode, cations.

It was noticed that the chemical change took place immediately the current was made, and as on measurement it appeared that Ohm's law held for electrolytes as much as for other conducting substances it was evident that the electrical energy expended in electrolysis is entirely used up in overcoming the resistance of the electrolyte, and that no work is done in pulling apart the components of the molecule. It followed that the ions existed in the solution previous to the passage of the current, and

hence the abnormal osmotic pressure of these conductors of electricity in solution, or electrolytes, received an explanation.

This ionisation of electrolytes is also described as electrolytic dissociation. It has been found that the order of the strengths of acids is the same as that of their electric conductivities in equivalent solution, and this is determined by the degree of dissociation. Since the hydrogen ions of any acid take a predominant share in conveying a current it is natural to suppose that the activity of acids is due to their common constituent, hydrogen ions. This assumption is in complete accord with the experimental facts. A strong acid, then, is one that dissociates largely, and a weak acid one that dissociates slightly at a given moderate dilution. Similarly the strengths of bases depend on the degree of their dissociation and the resulting concentration of the OH ions.

Now the common solvent, water, HOH, contains H and OH in union, and the question arises whether any dissociation of these occurs in pure water. A water absolutely pure is exceedingly difficult to prepare, but the purest so far obtained is found to conduct electricity slightly, and from the measurement of this, and by other indirect methods, it has been discovered that the concentration of the H ions, which is necessarily the same as that of the OH ions, is $1{\cdot}1 \times 10^{-7}$ at 25° C. In other words pure water contains by weight 1 gram of H ions and 17 grams of OH ions (the actual number of each kind of ions being the same) in 10,000,000 litres.

It follows from this that water can act both as a weak base and a weak acid. Regarding it as a weak acid it has been found that when two acids are allowed to compete for the same base the latter distributes itself between the acids in proportion to their avidities, and it has also been

shown that the ratio of their avidities is the ratio of the extent to which they are electrolytically dissociated. In aqueous solutions water, in virtue of its hydrogen ion concentration, can be regarded as one of the competing acids. In the case of a salt of a strong acid, such as sodium chloride, so weak an acid as water can take no appreciable amount of the base; hence in an aqueous solution of sodium chloride there are only Na and Cl ions and undissociated sodium chloride in appreciable amount, and the solution is neutral. If, instead, a salt of a weak acid and a strong base is taken, the conditions are different. Water is comparable in strength to a weak acid like hydrocyanic acid, and, therefore, a distribution of the base takes place between the acid and water according to the equation $KCN + HOH \rightleftharpoons KOH + HCN$. Since KOH is much more highly dissociated than HCN there are many more OH ions than H ions in the solution, and it is therefore alkaline. A similar argument applies to the salt of a weak base and a strong acid, where water as a base competes for the acid, and the acid so formed, being much more strongly dissociated than the hydroxide of the base, makes the solution acid. The taking up of the H or OH ions already existing in water destroys the equilibrium between them and the undissociated water, and, consequently, fresh ions must be formed by the splitting of hitherto undissociated molecules. This process goes on till a new equilibrium is established, taking in the OH ions present in the case of the strong base and weak acid, and the H ions in the case of the weak base and strong acid. In both cases the amount of hydrolysis is small, except the base or acid is very weak indeed. Sodium carbonate in 1 in 10 molar solution at 25° C. is hydrolysed 3·17 per cent., KCN 1·12 per cent., and sodium acetate, though acetic is a fairly weak acid, 0·008 per cent.

Also ammonium chloride 0·005 per cent., aniline hydrochloride 1·5 per cent., glycocoll hydrochloride 20 per cent., as glycocoll is a very weak base. The degree of hydrolysis is in both cases dependent on the dilution. When, however, the salt is one where both acid and base are weak, the degree of hydrolysis is independent of dilution and is considerable, *e.g.* aniline acetate about 44 per cent. Nevertheless hydrolysis does not always occur when it might be expected, *e.g.* sodium stearate is considerably hydrolysed, sodium palmitate is not.

With regard to the salts of strong acids and strong bases where no appreciable hydrolysis occurs a certain cautiousness of statement must be observed. It is true that the salts of the alkalies and alkaline earths are hydrolysed at ordinary temperatures to an inappreciable extent, but that hydrolysis does occur is indicated by Emich's experiment, where drops of water are applied to sodium chloride heated to redness. The drops are found to contain acid, and the salt left in the crucible after cooling is found when dissolved to give an alkaline reaction. This is in line with such facts as the appreciable hydrolysis of cane sugar by water at 100° C. and of salicin at 150° C. previously mentioned.

While the theory of hydrolysis just stated is the accepted one, it must be mentioned that Werner gives strong reasons for thinking that it does not explain the hydrolytic change in the aquometallic ammino salts, *e.g.* the aquopentammincobalti salt $\left[Co^{OH_2}_{(NH_3)_5}\right]X_3$, where X is an acid residue. He finds that it is due primarily to the stability of the water in the complex, and that this is the same thing as saying that it depends on the degree of strength with which the H ion, or hydrion, is linked. Reasoning from these compounds he believes that an

ordinary simple salt first takes up water, *e.g.* KCN becomes $\left[\begin{matrix}K\\H\end{matrix}O.H\right]CN$, and that this in neutral solution splits up into $\begin{matrix}K\\H\end{matrix}O$ and HCN. Then the bodies so formed ionise according to their strengths as acids or bases. KOH is strongly dissociated, and hence OH ions are present in the solution to a considerable extent, while HCN is very little dissociated, and hence few H ions are present; as a result the solution is alkaline.

The difference between the two theories may be stated as follows. Both theories put down hydrolysis to the action of water; but Arrhenius' theory makes hydrolysis dependent on the existing ions of water; Werner's theory makes it dependent on the previous association of the salt with undissociated molecules of water, which, when in combination with the salt, split, the metallic portion of the salt going with the OH half and the acid residue with the H half.

Obviously Werner's theory is based on the assumption that molecular combination between solute and solvent, when the latter is water, occurs. Evidence for this will now be considered.

(1) The solubility of gases in water is diminished both by electrolytes and by some non-electrolytes. Now the amount of gas taken up by a definite volume of liquid depends on (*a*) the pressure of the gas, (*b*) the temperature, (*c*) the nature of the gas, and (*d*) the nature of the liquid; (*a*), (*b*), and (*c*) remaining constant, the effect of solutes must be on the nature of the liquid. Were the solute evenly distributed throughout the liquid without combination there would be present the same amount of liquid as before the solute was added, and the same amount of gas should be taken up; but if combination of

some sort takes place between the solute and the water molecules the amount of liquid available for dissolving the gas would be diminished and the effect accounted for.

(2) The absorption of light by solutions of substances is by Beer's law proportional to the number of molecules through which the rays pass. If the absorption spectra of solutions of copper chloride of different degrees of concentration be examined, when the depth of the solution is varied in inverse proportion to the concentration so that the same total amount of solute lies in the path of the light, it will be found that more ultra-violet light is absorbed the more concentrated the solution is, although by Beer's law, the number of molecules remaining the same, the absorption ought to be the same. This might be accounted for by either of two assumptions: (*a*) that in the more concentrated solutions aggregates of the solute are formed which increase the absorbing power, or (*b*) that in the more dilute solutions aggregations of water and solute are formed, the amount of water taken up being in proportion to the dilution, and that these diminish the absorbing power. To test this, raise the temperature. The effect of this is to break up aggregates, whether of the solute molecules alone or of the solute molecules and water molecules. If the effect is to diminish the absorption, then probably the cause of increased absorption with increased concentration is aggregation of solute molecules; if, on the other hand, the effect is to increase the absorption, this is probably due to the breaking up of the combination between solute and water, so that the diminishing effect on the absorption of the combination is prevented. As a matter of fact, rise of temperature increases the absorption, therefore the diminished absorption on dilution is probably due to combination of water and solute.

(3) Kohlrausch has observed that the influence of temperature on the electrolytic resistance of dilute solutions of salts approximates to the temperature coefficient of the mechanical friction of water. This is intelligible if it is assumed that the ions are surrounded with water molecules, as the resistance to movement of the water-coated ions would then be the frictional resistance of water against water.

(4) The degree of dissociation of a solute can be determined by conductivity measurements, etc. This being known, the total amount of ionised and of undissociated salt is known, and from this the osmotic pressure can be calculated. Further, the depression of the freezing-point which ought to be produced by the addition of such an amount of the solute to pure water can be predicted. As a matter of fact, however, the experimental depression of the freezing-point is in many cases greater than it should be. If it be assumed that association of part of the solvent with the solute takes place, so that the amount of solvent present in the free state is diminished, then the solution will be more concentrated than was allowed for and the abnormal depression of the freezing-point accounted for.

(5) Kohlrausch found that the rates of migration of different ions approached nearer to the same value as the temperature was raised, and that at the critical temperature of water the mobilities would be identical. This can be accounted for on the assumption that at this temperature the ions are completely free from water, but that at lower temperatures some take up more molecules of water than others, and the bulkier the combination is the slower the movement.

(6) The behaviour of double salts in solutions appears to be the strongest evidence available, and it is on this

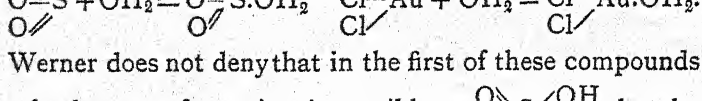
that Werner has founded his theory of valency of which the combination of water and solute molecules is only one section. Werner's theories are based on the fact that it is impossible to represent the structure of many double salts along the lines of ordinary valency formulæ. For example, while the structure of K_2SO_4 derived from the reaction represented by the equation $K_2O + SO_3 = K_2SO_4$ is thus shown $\begin{matrix} O \\ O \end{matrix}\!\!>\!S\!<\!\!\begin{matrix} OK \\ OK \end{matrix}$, it is impossible to represent a double halide in this form, yet there is clearly an analogy between the two

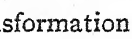
$$K_2O + SO_3 = K_2SO_4$$
$$KCl + AuCl_3 = KAuCl_4.$$

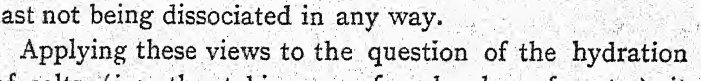
To represent the structure of $KAuCl_4$ in the ordinary manner involves conceptions of changes of valency on the part of Au or Cl. Similarly for other double salts it is necessary to assume so many changes of valency that it would almost appear that an element can unite with an indefinite number of atoms of another element. Now, it is true that up to a ecrtain point this is correct, *e.g.* four chlorides of vanadium have been isolated with two, three, four, and five atoms of combined chlorine respectively, but there seems to be a maximum for each element. This maximum can be determined by a study of the compounds of an element, but evidences of the variability of the valency of some elements are either not to be found or the facts can be explained in some other way. Chlorine can be represented as a monad in practically every case.

Werner believes that there is one cause underlying all such apparent difficulties of the theory of valency, which, being granted, an harmonious scheme becomes possible. The cause assumed is, that in addition to the usual principal valencies which unite atoms to atoms there also exist other valencies, which he calls auxiliary valencies,

and which are capable of uniting *molecules*. For example, to take the case which is of most immediate interest in this connection, water combines with oxides, chlorides, iodides, and many other classes of compounds, *e. g.*:

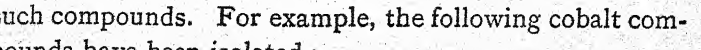


Werner does not deny that in the first of these compounds a further transformation is possible to  , but he points out that such a transformation is impossible in the second body. Similarly, $PtCl_4$ behaves like SO_3 in forming addition compounds with $2HCl$ and $2OH_2$, in both cases forming dibasic acids, and it also unites with $2NH_3$, yet in none of these cases does Cl appear as an ion. For this and other reasons he concludes that the Cl atoms are directly united to the Pt, *i. e.* they are, as he phrases it, in the first or non-dissociable zone of the attraction of the Pt atom, while the two H atoms, which are dissociated (in the first two combinations) are in the second or dissociable zone. He therefore formulates the compounds thus:



or $(PtCl_6)H_2$, $(Cl_4Pt(OH)_2)H_2$ and $(Cl_4Pt(NH_3)_2)$, the last not being dissociated in any way.

Applying these views to the question of the hydration of salts (*i. e.* the taking up of molecules of water) it appears that there is no reason why they should not form compounds with water in all cases, and that there is definite evidence that in many cases they do actually form such compounds. For example, the following cobalt compounds have been isolated:



X being a monobasic acid residue ; and many hydrates of metallic salts are known, of which he mentions those of chromium, stating that these have been thoroughly investigated.

$(Cr(OH)_6)Cl_3$.

$\left[Cr^{Cl}_{(OH_2)_5}\right]CL_2 + HOH.$ $\left[Cr^{Cl_2}_{(OH_2)_4}\right]Cl + 2HOH.$

Many metallic salts form hydrates with six molecules of water, *e.g.* $(Ca(OH_2)_6)\ Cl_2$, $(Fe(OH_2)_6)Cl_3$. Six appears to be the maximum number of water molecules with which a salt can unite, at any rate in the first zone of attraction to the central atom ; when this occurs all the acid residues in the salt are ionised. Water can also be bound in the second zone, as in the case of the sulphates, which can take up seven molecules of water, six in the first zone, one in the second.

Acids, as well as salts, can form hydrates, thus the following have been isolated: $NO_3OH.OH_2$, $NO_3OH.3OH_2$, $HCl.2OH_2$, $HCl.3OH_2$. To explain such a compound as $HCl.OH_2$ (which has not actually been isolated, but to which analogies exist in organic chemistry), the theory of the quadrivalency of oxygen has been elaborated, thus $\begin{matrix}H\\H\end{matrix}\!\!>\!O\!<\!\!\begin{matrix}H\\Cl\end{matrix}$, but it is impossible to apply this to compounds with two or more molecules of water except on the assumption of the existence of chain compounds, *e.g.* $\begin{matrix}H\\H\end{matrix}\!\!>\!O\!<\!\!\begin{matrix}H\\H\end{matrix}\!\!>\!O\!<\!\!\begin{matrix}H\\Cl\end{matrix}$, and chain formulæ for the hydrates of metallic salts, for the metallic ammino-complexes, etc., to-day are accepted universally as incorrect (Werner). It is simpler to assume that hydrogen can act as a central atom, and the formulæ become :

$$ClH.OH_2,\ ClH:\begin{matrix}OH_2\\OH_2\end{matrix},\ \text{etc.}$$

On these general grounds Werner comes to the con-

clusion that in solution in water a salt (where Me = metallic radicle and X = acid residue) should be represented by the formula $\left[\begin{matrix}Me\\H\end{matrix}\!\!>\!O.H\right]X$. If only electrically dissociated this becomes $\left(\begin{matrix}Me\\H\end{matrix}\!\!>\!O.H\right)^{+}$ and X^{-}, but if hydrolysis precedes electrolytic dissociation the bodies present will be H^{+}, X^{-}, and $\left(\begin{matrix}Me\\H\end{matrix}O\right)$ not or little dissociated if a weak base, and the solution will be acid. If HX is a weak acid and MeOH a strong base the bodies present will be Me^{+}, OH^{-}, and HX not or little dissociated, and the solution will be alkaline.

Werner's hypothesis that all salts in solution, being combined with molecules of water, hydrolyse by separation at the junction of the H and OH of the water, if correct, affords an explanation of the effect of precipitating a negatively charged suspensoid colloid, such as arsenious sulphide, by the adsorption of ions of opposite sign, *e. g.* the barium ion of barium chloride. It has been shown in this case that the Ba ions go down with the precipitate, while the liquid becomes acid owing to free hydrochloric acid. The difficulty is the disposal of the Cl ions. Even if we suppose that more water is dissociated to give the increase of H ions shown by the acid reaction there still remain the corresponding OH ions to be accounted for (Bayliss). This difficulty arises from assuming that the ions formed are Ba^{++}, Cl^{-}, and Cl^{-}. But if it is assumed in accordance with Werner's theory that the salt was hydrated before it exerted its action, then it would have the form $\left[Ba\begin{matrix}OHH\\OHH\end{matrix}\right]\begin{matrix}Cl\\Cl\end{matrix}$, and if this merely ionised the ions would be $\left[Ba\begin{matrix}OHH\\OHH\end{matrix}\right]^{++}$, Cl^{-}, and Cl^{-}, and no doubt in this form it reaches the colloid. Now it is clear

that adsorption exerts a chemical action on the salt, for the solution becomes acid, and if this chemical action is granted to be a hydrolysis of the Werner type, the whole would split at the junction of the H and OH radicles of water, and as a result $Ba(OH)_2$, H^+, H^+, Cl^-, Cl^-, would be present. What finally unites with the colloid is the $Ba(OH)_2$, which in virtue of its union does not dissociate, at any rate in the solvent, while hydrochloric acid is set free. Whether this explanation is accepted or not, one thing is clear, that hydrolysis of the salt has taken place, and this is much more easily explained as a result of adsorption of the salt than as due to the ions of water already existing in the solvent.

Werner's theory deals only with chemical combination of water in other molecules, although of an unusual kind, he leaves untouched the question whether association of still another kind occurs between the solute and the solvent, and whether also the ions themselves are associated with particles of the solvent. The evidence previously given is in favour of this additional association.

There is, therefore, reasonable ground for believing that, in the case of water at any rate, molecular combination between solute and solvent occurs, though it must be stated that Nernst considers the hypothesis by no means proven.

Apart from all theories, however, since in any case of hydrolysis the result is brought about by the union of an H radicle with one of the parts into which the solute divides, and of an OH with the other, it is clear that the hydrogen ion concentration must be a matter of great importance. (The H ion concentration gives also the OH concentration, since the product of the two is a constant.) In fact the rate at which the esters, methyl and ethyl acetate, are hydrolysed in water is found to be pro-

portional to the H ion concentration present. Since both H and OH ions cannot be present in equal concentration at the same time except at the point of neutrality when the concentration of either is extremely low at ordinary temperatures, and since the increase of one means the proportionate decrease of the other, it follows that any hydrolytic process can be carried out in connection with one of the two only, the other coming into existence secondarily as required. That one ion is sufficient appears from the following scheme. Assume the existence of a free OH ion. This meeting with a substance XY will, if its attraction for Y is stronger than that of X, form YOH, leaving X^- free as an unsatisfied ion. This can either break the union of H and OH in a molecule of water, or, perhaps more probably, it unites with an H ion already present in the solvent. This leaves a fresh OH ion to carry on the action.

$$OH^- + XY = YOH + X^-$$
$$X^- + H^+ + OH^- = HX + OH^-.$$

Similarly if an H ion is assumed free,

$$H^+ + XY = HX + Y^+$$
$$Y^+ + H^+ + OH^- = YOH + H^+.$$

Of course the existence of a free ion would be only momentary. The action of the H or OH ions must start before they are freed from their electrostatic attraction, and must depend on the superior attraction of Y or X for them over the attraction exercised on them by the radicle with which they are combined.

Now if H and OH as ions can produce hydrolysis there ought to be evidence that water, which contains them and them only, can act as a powerful hydrolyser provided the concentration of the ions can be raised to any considerable extent. In water at 0° C. the concentration is very low, $0{\cdot}35 \times 10^{-7}$, but it rises rapidly with the temperature.

At 18° C. it is $0{\cdot}80 \times 10^{-7}$, and at 50° C. $2{\cdot}48 \times 10^{-7}$. That the hydrolysing action becomes evident in water when the temperature is sufficiently raised is shown by its action on glucose at 100° C., on salicin at 150° C., and on red-hot sodium chloride, when the water is in the spheroidal state, as already mentioned. It follows, therefore, that if any method can take the place of raising the temperature in increasing the concentration of the H and OH ions in an aqueous solution, either generally or at the point of action, this method must be indirectly a powerful hydrolyser. If then acids, bases, or salts can increase the concentration of either ion generally, and enzymes can increase it at the point of action, viz. the surfaces of their particles, they themselves being either unchanged or else reformed in the course of the reaction, these bodies will indirectly be hydrolysers and will conform as regards hydrolysis to the definition of catalysts.

Summary.

(1) Many different enzymes are known, but their chemical action on their substrates in most cases is hydrolytic.

(2) Hydrolysis is a kind of reversion of the process of neutralisation of an acid by a base, or *vice versâ*. Unless both acid and base are weak the amount of hydrolysis is small, and if both are strong the amount is insignificant.

(3) Hydrolysis belongs to the class of reversible reactions.

(4) In some cases simple solution in water is sufficient to produce hydrolysis, but in other cases the action is very slow and may be hastened by catalysts.

(5) The action of enzymes on the three great classes of food-stuffs, the fats, the carbohydrates, and the proteins, is hydrolytic.

(6) The same is true of enzyme action on some simpler bodies, such as urea, the amino-acids, and the purine bodies.

(7) Processes accompanied by coagulation are, so far as the coagulation is concerned, colloid reactions, and are not enzymic.

(8) On the assumption that in certain decompositions produced by enzymes there is formed an intermediate compound combining water in an unstable form, these processes also appear to be hydrolytic.

(9) Fermentation is a complex process involving a series of reactions, but all these processes can be represented as hydrolytic and oxidative, through hydration and its opposite, with accompanying rearrangement of the H and OH radicles.

(10) An electrolyte is a substance which when dissolved can conduct a current of electricity. It exists in the solution partly undissociated, and partly as electrically charged radicles. These charged radicles, called ions, are the real conductors of the electric current.

(11) The facts on which the last statement is based are given. They include the abnormal osmotic pressure of electrolytes, their conductivity, and their obedience to Ohm's law.

(12) The order of the strengths of the acids is the same as the order of their electrical conductivities, and this is determined by the degree of their dissociation. Hydrogen ions are the common constituent of all acids, and it is to these that their activity is due. Similarly the activity of bases is due to their hydroxyl ions.

(13) In the purest water there is a certain small amount of dissociation so that both H and OH ions are present. Water can, therefore, act both as a weak base and a weak acid.

(14) The theory of Arrhenius attributes hydrolysis to the existence of these ions in water, and the facts of hydrolysis in the four possible cases, salt of a strong acid and a strong base, of strong acid and weak base, of weak acid and strong base, and of weak acid and weak base, can be accounted for on this theory.

(15) Werner has pointed out that the theory is not applicable to some complex salts, and on this account he has formulated a theory which attributes hydrolysis to previous hydration of the salt and subsequent division of the complex at the junction of the H and OH radicles of the water so taken up.

(16) Werner's theory assumes that molecular combination between solute and solvent occurs. The facts bearing on this are: (1) The effect of electrolytes, etc., on the solubility of gases in water; (2) the effect of concentration or dilution of a solution on the absorption of light when the number of molecules in the path of the rays is kept constant; (3) the influence of temperature on the electrolytic resistance of dilute solutions of salts; (4) the abnormal depression of the freezing-point in certain salt solutions; (5) the rates of migration of different ions as depending on the temperature; (6) the behaviour of double salts in solution.

(17) A *résumé* of Werner's theory of valency is given so far as it bears on hydration and hydrolysis. He gives evidence of the existence of complex salts which include water combined in their molecules, also of the existence of combinations of water with acids.

(18) The disposal of the acid radicle in the case of the precipitation of a suspensoid colloid by a divalent positive radicle like barium with combination of colloid and positive radicle is explained on Werner's hypothesis.

(19) Since hydrolysis involves the union of H and OH

radicles with the parts into which the hydrolysed substance divides, it follows that the concentration of the hydrogen ions in the solution is a matter of great importance.

(20) A scheme is given showing that the presence of either the H or the OH ion is sufficient to account for hydrolysis.

(21) The effect of temperature on the hydrolytic power of water can be traced to the change in the concentration of the H and OH ions so produced.

(22) If inorganic catalysts and enzymes can take the place of a rise of temperature in this respect their action as hydrolysers could be explained.

Chemical Action (continued). Oxidation and the Oxidases.

The processes of oxidation and reduction are not merely opposite, they are also complementary, for except an oxidation takes place in the presence of free oxygen, *e. g.* in air, the oxygen required for the oxidation must be derived from some substance containing it, and this latter substance, in virtue of giving up its oxygen, is reduced at the same time as the oxidation takes place. Probably even oxidations in air are not direct.

Oxidation of saturated substances, either in the laboratory or in the living organism, usually consists in the replacement of hydrogen atoms by hydroxyl groups. An example of this is furnished by the combustion of a hydrocarbon. Ordinarily a hydrocarbon burned with an adequate supply of oxygen yields nothing but carbon dioxide and water without any indication of the formation of intermediate compounds, but it has been shown by suitably modifying the conditions that unstable hydroxylated molecules are formed which subsequently undergo decom-

position into simpler products. For example, formaldehyde and steam may be detected in the combustion of methane, and the changes which occur are believed to be as follows : $CH_4 - CH_3OH - CH_2(OH)_2$, which on loss of water $- CH_2O$ (formaldehyde) $- (H_2 + CO) - (H_2O + CO_2)$. A similar change occurs in the oxidation of an aldehyde to an acid :

$$CH_3C\overset{H}{\underset{O}{}} - CH_3C\overset{OH}{\underset{O}{}}.$$

The origin of the OH groups in such cases is to be traced to the presence of water, whether in the form of liquid or vapour, for thoroughly dried carbon monoxide does not burn in thoroughly dried air, but when a trace of moisture is present combination takes place at once. Pure metallic sodium retains its bright colour in an atmosphere of dry oxygen, but is instantly tarnished the moment that a trace of moisture is admitted. On the other hand in perfectly pure air-free water, metals like zinc and iron retain their lustre undiminished, but if air is admitted they soon rust.

When ozone was discovered and its extremely powerful oxidising properties became known, it was suggested that all oxidations were due to its formation. That the formation of ozone sometimes occurs is shown by its occurrence during the slow spontaneous oxidation of phosphorus in air. But the oxidating substances produced in living cells have an oxidation potential which is not so high as that of ozone ; the latter liberates iodine from potassium iodide with great rapidity, the former very slowly. The theory that ozone is the active agent in all oxidations cannot be maintained, but outside the organism it may play a minor part.

Oxygen, however, in the molecular form, as in air, is a very slow oxidiser, and it is necessary, therefore, to account for the rapid oxidations which occur of substances which

when exposed to molecular oxygen remain practically unoxidised. In some way the oxygen must be rendered active.

Now it has been proved beyond doubt that if two oxidisable substances are exposed to oxygen, one of which is easily oxidisable and the other is not, during the oxidisation of the first the second also will become oxidised, although by itself it would remain unaffected. If sodium sulphite in solution is shaken with air it is rapidly oxidised; if sodium arsenite is similarly treated no effect is produced; but if both together are shaken with air *both* are oxidised. Such a reaction is called a "coupled" reaction. The active substance in this case oxygen, is called the actor, the readily oxidised substance the inductor, and the third, or difficultly oxidisable substance is known as the acceptor.

To account for the facts of oxidation there have been three main theories.

(1) The ionisation theory of Van't Hoff assumes that very small amounts of atomic (ionic) oxygen are constantly being produced from molecular oxygen, and that it is with these that the oxidisable substance enters into combination. That such gas ions exist is rendered probable by the facts of the conduction of electricity in gases, and the condensation of aqueous vapour in the steam jet as the result of the oxidation of various substances in close proximity, but only while the process of oxidation is going on. Van't Hoff believed that oxygen dissociated into positive and negative ions, and that the readily oxidisable—or "autoxidisable"—substance took one kind and the oxidisable substance the other. The facts, however, can be explained on theories of more general application.

(2) Hoppe Seyler's theory. Hoppe Seyler believed that oxygen is endered active by nascent hydrogen. Hydrogen

is liberated in many processes of anærobic fermentation in an active form, and he believed that it attacked molecular oxygen, appropriating one atom and leaving the other free to unite with an oxidisable substance, if present, if not, then with water forming hydrogen peroxide, HOOH. That nascent hydrogen is capable of effecting oxidations as well as reductions is shown by the fact that hydrogen absorbed by palladium can oxidise benzene to phenol, and toluene to benzoic acid in the presence of water and oxygen. The weak points of the theory are, according to Kastle, that it does not take into account the formation of other peroxides known to be formed besides hydrogen peroxide, and that it accounts for the latter by the oxidation of water. Kastle states that HOOH is never produced by the action of oxygen on water, even under the influence of light and heat, and in the presence of dilute sulphuric acid; but this is not strictly accurate, as ultra-violet rays will decompose water into hydrogen peroxide and hydrogen. Nevertheless it appears to be true that HOOH is not formed by the oxidation of water as a rule, but is formed as a secondary product of the hydrolysis of another peroxide, or sometimes by the oxidation of a labile hydrogen atom or ion of some compound.

(3) The peroxide theory founded by Traube and elaborated by Engler and others, depends on the fact that oxidations cannot proceed in the total absence of water. Traube believed (*a*) that the oxygen molecule *as a whole* combines with the oxidisable substance or with the hydrogen of water to form a peroxide; (*b*) that water actively participates in these autoxidations and that HOOH is formed as a primary product. For example, the oxidation of zinc in air and dilute sulphuric acid results not merely in its own oxidation but in the formation of a peroxide, HOOH, as well.

$$Zn + \begin{matrix} HO.H \\ HO.H \end{matrix} + O_2 = Zn(OH)_2 + HOOH.$$

He also believed (*c*) that the peroxide formed in this coupled reaction acts as a carrier of oxygen to the acceptor, and (*d*) that water is decomposed in oxidations with the formation of atomic hydrogen, and that this combines with molecular oxygen to form HOOH.

An objection to the theory is that it has not been found possible to prove the presence of HOOH in all oxidations; but then it may have only a momentary existence.

In view of the objections raised the theory has been somewhat modified, and may be stated thus: Molecular, or possibly ionic oxygen, unites with an autoxidisable substance producing a very unstable higher oxide, possibly by molecular combination, which immediately breaks down or is rearranged into a lower oxide and a peroxide, and it is the peroxide which, either with or without the intermediate formation of HOOH, acts as a carrier of oxygen to the acceptor, the peroxide in giving up its extra oxygen becoming the lower oxide. That this is more than a mere theory has been proved by the facts of the oxidation of indigo in the presence of benzaldehyde, C_6H_5CHO. The ultimate products are benzoic acid and oxidised indigo, but it has been shown that benzoyl hydrogen peroxide, $C_6H_5CO.O.OH$, is formed as an intermediate compound. Further, whatever may occur in the laboratory, the action of a peroxide in the living organism is the only hypothesis which will account for the fact than many of the oxidation processes which take place *in vivo* can be imitated *in vitro* only by hydrogen peroxide; *e.g.* the oxidation of the normal saturated fatty acids in the β-position;

$$CH_3.CH_2.CH_2.COOH \rightarrow CH_3CO.CH_2.COOH$$

Butyric acid. Acetoacetic acid.

and indole is oxidised to indoxyl in the body, but outside the body by HOOH only.

Most peroxides are hydrolysed in water in two stages, thus:

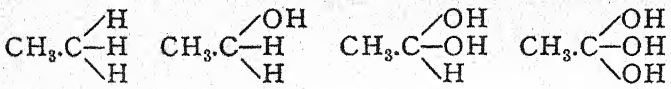
$$A\begin{cases}O\\|\\O\end{cases} + \begin{matrix}H\\OH\end{matrix} = A\begin{matrix}OH\\O.OH\end{matrix} = AO + HOOH.$$

Therefore a peroxide arising by autoxidation of an oxidisable substance produced by a cell gives rise, as a rule, to the formation of hydrogen peroxide.

There is evidence, however, that HOOH is not the peroxide concerned. The fresh juice of *Lathræa squamaria*, which contains an oxidase, was exposed to a current of air. This ensures the formation of a peroxide, if none was actually present in the fresh juice. If one be present it can be separated and precipitated by barium hydroxide, and as a matter of fact a precipitate is thus produced. The precipitate is filtered off and decomposed by sulphuric acid to set free the peroxide from its combination with barium. The proof that a peroxide is present is the blue coloration produced by adding the solution to a mixture of potassium iodide and starch. This peroxide is not HOOH, for if so it would give an orange-yellow colour with titanic acid, and it does not give this reaction. Nitrites can be proved to be absent; these would equally give the blue coloration with potassium iodide and starch. It follows that a peroxide is present which is not HOOH, and it is practically certain to be an acylhydroperoxide. That such a substance could be activated by peroxidase is proved by the action of the latter on ethyl hydrogen peroxide.

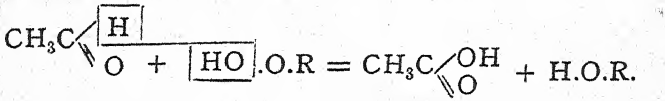
Apart from this there is also no necessity to assume the formation of hydrogen peroxide to account for the oxidations which occur in the organism. It has been pointed

out that these are replacements of H by OH, and it follows that in the oxidising substance there is a complementary replacement of OH by H. First, as regards the replacement of H by OH, the formation of alcohols, of aldehydes, and of acids can be represented as successive replacements of H by OH, thus:



Ethane.	Ethyl alcohol.	Ethyl aldehyde.	Acetic acid.

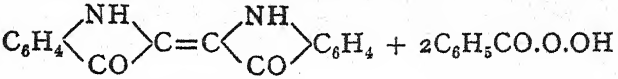
The forms shown lose a molecule of water where more than one OH radicle is attached to one carbon atom. Second, as regards the complementary replacement of OH by H, the example of a superoxide like HOOH or $C_6H_5CO.O.OH$ may be taken. In general terms the formula is R.O.OH, in the first case R = H, in the second R = C_6H_5CO.



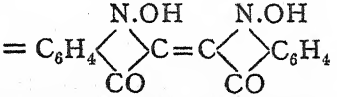
Aldehyde. Acetic acid.

H.O.R. or ROH is in the first case water, in the second C_6H_5COOH, or benzoic acid. If this be a correct statement of the case it is not the oxygen connecting the radicle R with OH which leaves its combination with R, but the OH itself. It is true that superoxides in general readily give off oxygen on treatment with dilute acid, but they do this not directly, but probably after first forming hydrogen peroxide. $BaO_2 + H_2SO_4 = BaSO_4 + HOOH$. Since the oxidation can be perfectly well represented as an exchange of H and OH between the reacting substances it is unlikely that an intermediate stage involving the giving off of oxygen is inserted. Further, of the two radicles, O and OH, in R.O.OH, it is on the face of it

probable that the OH is the more weakly attached of the two, for the attachment of OH, itself negative, is to a negative radicle, whereas O is in part attached to a positive one. Oxidation, therefore, by a superoxide like HOOH, the type that occurs in the living organism, and therefore the type which is *a priori* probable in any enzyme reaction, can be resolved into an exchange of places by the H and OH radicles. To account for all the cases, however, it is necessary to include a subsequent rearrangement of the atoms of the reacting molecules. This appears to occur with indigo when oxidised by $C_6H_5CO.O.OH$.



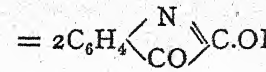
Indigo.



Hypothetical intermediate compound.

$+2C_6H_5COOH$. Benzoic acid.

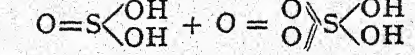
Hypothetical intermediate compound



Isatin.

The rearrangement breaks the double bond.

The theory of oxidation as a replacement of H by OH applies to the saturated compounds, but not to the unsaturated. For example, sulphurous acid, H_2SO_3, becomes sulphuric acid, H_2SO_4, apparently by the simple addition of oxygen:



and if SO_2 mixed with oxygen is passed over platinised asbestos at 400° C. SO_3 is formed. In the latter case it is

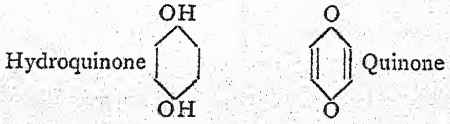
not clear that the gases are thoroughly freed from traces of moisture, while in the former the reaction takes place in aqueous solution. Further, the oxidation of sulphurous acid by chlorine and its reduction of iodine to hydriodic acid include the action of water. The reaction with chlorine may be represented as follows:

$$O{=}S\begin{matrix}OH\\OH\end{matrix} + 2HOH + Cl_2 = \begin{matrix}O\\OH\\OH\end{matrix}{-}S\begin{matrix}OH\\OH\end{matrix} + 2HCl$$

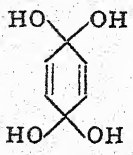
$$= \begin{matrix}O\\O\end{matrix}{>}S\begin{matrix}OH\\OH\end{matrix} + HOH + 2HCl.$$

It follows that the oxidation of an unsaturated compound is due, not to the replacement of H by OH, but to the addition of OH, and that the OH required can be obtained from water, provided that the hydrogen radicle of water has first been removed.

Such a reaction occurs with enzymes in the oxidation of the polyphenols. Catechol, ortho-, and resorcinol, meta-dihydroxybenzene, in alkaline solution are autoxidisable in air, but hydroquinone, or quinol, the para-compound, is not; it is easily oxidised by laccase to quinone. Quinone is not a benzene derivative; it is a cyclic diketone containing two double bonds, the para-diketone of a dihydroxybenzene.



The reaction probably results in the addition of OH thus:



Since each carbon atom in benzene can unite with only one monad atom the formation of this double hydroxide

involves the disappearance of the benzene structure, two bonds which were formerly internal having become external, and the four remaining bonds unite in two pairs as shown. As usual, the combination of carbon with more than one OH is unstable and HOH separates, leaving:

Quinone

$$\text{or } HO.C_6H_4.OH + HOOH = \begin{matrix} HO \\ HO \end{matrix} C_6H_4 \begin{matrix} OH \\ OH \end{matrix}$$
$$= OC_6H_4O + 2HOH.$$

The HOOH represents the peroxide supplied by laccase.

Since a superoxide is concerned, it follows that the characters of any superoxide oxidation are to be found with the whole group of superoxides, hence reactions which in the organism may be carried out by organic peroxides can be carried out in the laboratory by hydrogen peroxide, and in experimenting with the oxidases it is legitimate to use HOOH as the peroxide, whether this is the actual peroxide used by the cells or not.

A difficulty may be felt here from the point of view of catalytic or enzyme action. Since the formation of the peroxide implies the simultaneous formation of the lower oxide, and itself in giving up its oxygen or hydroxyl becomes converted into the lower oxide, the previously autoxidisable substance now exists in the form of a lower oxide, *i. e.* it is no longer autoxidisable, its special quality has disappeared. There is evidence that this occurs in oxidase action, for Kastle and Loewenhart and also Bach have pointed out that the oxidases are not true ferments or oxygen-catalysts, because they are unable to accomplish the oxidation of practically unlimited amounts of oxidisable material. This is intelligible if a peroxide is concerned in the process, since it cannot be reformed from the same material. There is, however, evidence that

autoxidisable substances exist which can be reformed on parting with their oxygen. Hæmoglobin is the best known case. Since its absorption of oxygen appears to have relation both to its chemical constitution and to the tension of the oxygen to which it is exposed, Bayliss suggests that both chemical and adsorption combinations are concerned, but from the point of view of enzyme action hæmoglobin does not enter into the question except as the means of providing oxygen for oxidases, and this seems not to occur through enzyme action, for the oxygen might be equally well supplied to the enzyme by shaking its solution with air. If the oxidases cannot oxidise unlimited quantities of material, and if the peroxides, by means of which they act, cannot be reformed from the same material, it would appear that enzyme action in the process of oxidation is excluded. There is evidence, nevertheless, that it does occur, and this will now be considered.

The oxidases, or oxidising enzymes, which are known are very few, though there are indications of the existence of others. They are: (1) Laccase, originally found in the juice of the lac tree, an enzyme which, when the juice is allowed to stand in air, gradually turns it black; the boiled juice does not show this change, nor does the change occur in the absence of oxygen. (2) Tyrosinase, which turns tyrosin black, forming the melanin pigments. (3) Indophenol oxidase, which oxidises a mixture of *a*-naphthol and paraphenylene diamine to indophenol, a blue compound. (4) The purin oxidases, which convert xanthin and hypoxanthin to uric acid, and in some cases oxidise uric acid itself. (5) Catalase, which acts specifically on HOOH, producing molecular, *i. e.* inactive, oxygen. It is, properly speaking, not an oxidase. Aldehydase is sometimes included, but is rather to be classed with the

reducing enzymes. Of these, laccase, or enzymes acting in the same way, is almost universally distributed in the plant world, but is found in certain animal cells only. Tyrosinase is widely distributed among plants, but not so widely as laccase; it is, however, much more abundant in the animal kingdom, where it plays an essential part in the formation of animal pigment.

Laccase may be taken as the type of oxidising enzymes, as its method of action appears to be common to the oxidising enzymes, though minor differences are to be found, especially in the influence of acids and alkalies and of hydrogen peroxide on each.

The reaction by which the presence of laccase or laccase-like enzymes is recognised is the guaiacum blue reaction. The gum-resin guaiacum contains guaiaconic acid, and it is this which on oxidation gives the blue colour.

The fundamental experiments are:

(1) To a scraping from the surface of a potato apply a drop of alcoholic solution of guaiaconic acid: a blue colour is produced. The same effect is produced by scrapings from the root of the horse-radish and many other plants.

(2) Hydrogen peroxide does not give this blue colour with guaiaconic acid.

(3) A solution can be prepared from the root of the horse-radish by a complicated method (principally by the use of alcohol), which with guaiaconic acid does not give the blue colour.

(4) A mixture of this preparation, hydrogen peroxide, and guaiaconic acid gives the blue reaction.

(5) If in the first experiment, before guaiaconic acid is added to the potato scrapings, the tube containing the scrapings is exposed to a current of hydrogen or any

neutral gas till the oxygen is wholly displaced, a blue colour is no longer obtained on addition of guaiaconic acid.

From these experiments it is inferred that a peroxide like hydrogen peroxide must be present, and that it is this which oxidises the guaiaconic acid, but not until it is activated. The activator, because it acts on peroxides, is called peroxidase, and is the true enzyme of oxidation. It has the general properties of an enzyme, for it is destroyed by heat and it can act on practically unlimited quantities of the peroxide provided that an oxidisable substance is present and that this is removed when oxidised. In contact with HOOH alone it is gradually destroyed. In all these points it acts like a true enzyme.

It is now clear why oxidases did not appear to be true enzymes. Oxidases like laccase, tyrosinase, etc., are really compound bodies consisting of the true enzyme peroxidase, a peroxide, and, it may be, an autoxidisable substance. Their defects as enzymes are due to the peroxide constituent, for this must be gradually used up in the course of the reaction.

The oxidases in general appear to be less specific in their action than the hydrolytic enzymes. Laccase oxidises not only laccol but also guaiacum, guaiacol, hydroquinone, phenolphthalin, adrenalin, and a large number of phenols and aromatic amino-compounds. There is a certain specificity, however, as the only substances easily oxidised by laccase are those of the benzene series containing hydroxyl or amino groups in the ortho- or para-positions. Tyrosinase acts most readily on the homologues of phenol in which the side chains occupy the para-position.

Like the hydrolytic enzymes the oxidases are sensitive to the action of acids, alkalies, and salts. Laccase is

poisoned or paralysed by most acids, at any rate beyond a very low concentration, but some, such as carbonic, boric, and phosphoric, have no effect at any concentration. Hydrocyanic acid and hydrogen sulphide, hydroxylamin, phenyl hydrazin, and similar reducing bodies destroy the action of all oxidases. Tyrosinase is inhibited by N/100 HCl and is considerably retarded by N/100 NaOH. Neutral salts in general retard its action.

There are also certain salts which, like the co-enzymes of the hydrolytic enzymes, are either absolutely necessary to the action of the oxidases or very greatly stimulate their action. Bertrand found that laccase always contains small amounts of manganese, and that its oxidising power is proportional to the amount of manganese present; further, that laccase oxidations are greatly accelerated by small amounts of manganese salts, and that no other metal is capable of taking its place. The tyrosinase found in the skins of animals acts upon tyrosin only in the presence of small amounts of iron. Slowtzoff claims to have prepared a laccase free from manganese, but, on the other hand, Bertrand showed that a specimen poor in manganese was comparatively inactive, and that the effect of the addition of manganese to this preparation was to increase its oxidising power twenty-five times. Manganese salts by themselves can bring about the oxidation of various oxidisable substances, such as hydroquinone, in air, and the quantity of oxygen absorbed varies with the nature of the manganese salt, being greater with the salts of the organic acids. It appears, therefore, that those manganese salts which are most easily hydrolysable are the most efficient oxygen carriers. It is clear, therefore, that the condition of the manganese salt itself is important, and that the active state is the hydroxide. Iron salts have a similar action.

Schinoxidase from the latex of *Schinus molle* gives the same reactions as laccase, but its ash contains no manganese and is rich instead in iron. Again, ferrous salts in extremely small amounts strongly accelerate the action of HOOH on oxidisable substances. Ferrous salts, too, as also those of copper and manganese, accelerate the oxidising powers of oil of turpentine, benzaldehyde, etc., no doubt by action on the peroxides produced. This is in line with the dependence of the oxygen-carrying power of hæmoglobin on its iron content, for the guaiacum test for blood, which is an indication of oxidising power, is given even by the iron-containing derivative hæmatin, but not by the iron-free hæmatoporphyrin. In the molluscs and crustacea hæmocyanin, a copper-containing substance, acts like hæmoglobin as an oxygen carrier. Zinc is found in the blood of certain marine forms, and salts of zinc in extraordinary dilutions have a great effect on the growth of moulds.

Peroxidase is the most widely distributed of enzymes. It can certainly be said of any living tissue or organ that it is dead when it fails to show the reactions of the peroxidases. It is also extraordinarily resistant to decay. All seeds which retain their germinating power contain peroxidases, and in corn they have been recognised in samples over two hundred years old. Peroxidase outlasts the power to germinate by a hundred years. It therefore appears that peroxidase activity is one of the most characteristic and persistent properties of living material.

Catalase is a remarkable enzyme. Loew has arrived at the conclusion that there does not exist a cell which does not contain some catalase. Its action is exclusively on hydrogen peroxide, which it decomposes with the formation of molecular, *i. e.* inactive, oxygen. In this respect it is unique, for all other substances which decompose

hydrogen peroxide activate the oxygen, *e.g.* even finely-divided platinum. The simplest explanation of this action is that it always reacts with two molecules of HOOH and never with one. $2HOOH = 2HOH + O_2$. As it does not activate oxygen it is not an oxidase proper, though its enzyme action is undoubted. It is believed to exert a protective action against hydrogen peroxide formed as a by-product, and possibly acts also as a regulator of peroxidase action, for in a mixture of HOOH with peroxidase and catalase active oxygen and molecular oxygen are formed in proportion to the relative amounts of the two enzymes.

Reduction and the Reducases.

There appears to be some doubt as to the existence of these enzymes. Euler says that most apparent cases of reducase action are really stoichiometric reductions by a readily oxidisable substance, and that catalytic action is not concerned. The following experiments by Schardinger seem, however, to be conclusive :

(1) Fresh milk has no action on methylene blue.

(2) Fresh milk reduces methylene blue to the leuco or colourless base if an aldehyde such as acetic or formic is present.

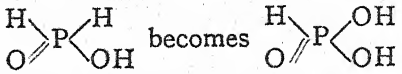
(3) Boiled milk has no action in the presence of an aldehyde on methylene blue.

(4) The action of bacteria in the milk can be excluded.

Reductions in general are due to the action of nascent or active hydrogen, and the source of this is of importance.

Analogies with reducase action have been found in the action of the platinum group of metals which give the key to the source. If a hypophosphite is dissolved in water

no change takes place, but if finely divided palladium is also present it is oxidised.



The oxygen which enters the compound in the form of OH can come only from the water of the solution. Thus: $H_3PO_2 + 2HOH = H_3PO_3 + H_2 + HOH$. The hydrogen represented as being evolved is absorbed by the palladium and is later set free. If an aldehyde is taken in place of the hypophosphite the presence of metals of the platinum group has no effect, but if an acceptor for the nascent hydrogen is also present the aldehyde is oxidised to the corresponding carboxylic acid. Acceptors are methylene blue, nitrates, indigo, etc., all easily reducible substances, but none of them is reduced at any perceptible rate by formaldehyde alone without platinum. Since methylene blue contains no oxygen, the oxygen used in the oxidation of the aldehyde must come from the water of the solution.

R.CHO + HOH + meth. blue = R.COOH + H. meth. blue., *i. e.* reduced.

The enzyme which appears to be contained in fresh milk takes the place of finely divided platinum in the above reaction.

To explain the reducing action of milk, etc., Bach has suggested the existence of a perhydride of oxygen in water, H_4O, on the ground that oxygen may be quadrivalent, and on analogy with the action of peroxidase on peroxides he has suggested the name, perhydridase, for the milk enzyme. Similar reducing enzymes have been described in the tissues, whose powerful reducing action while fresh is well known, and a reducing enzyme has been extracted from the liver which reduces methylene blue in the presence of an aldehyde.

From the point of view of the oxidation of the aldehyde the enzyme has been described as an aldehydase. It appears to be independent of the presence of atmospheric oxygen, for liberal supplies of fresh oxygen diminish the rate of oxidation and may even suppress it altogether. The process resembles anaerobic fermentation where the oxygen required is furnished not by the air but by oxygen-containing compounds, in this case, water.

Bach's assumption of a perhydride in water seems to me totally unnecessary. It has already been pointed out that any oxidation involves a reduction, in general of a peroxide, but in the case of anaerobic fermentation the reduction must be of some substance containing oxygen and not a peroxide. Now, as an oxidation involves a reduction, so a reduction must involve an oxidation, and while the action of a reducing enzyme is reducing on the substance regarded—in the case under consideration, methylene blue—it is equally an oxidising enzyme from the point of view of the substance oxidised, viz. aldehyde. Treating it then as an oxidising enzyme, it derives its oxygen from the water of the solution, and the oxidation conforms to the usual type of oxidations in being a replacement in the aldehyde of H by OH:

$$CH_3C\begin{cases}\boxed{H}\\ O + \boxed{OH}\end{cases}H = CH_3C\begin{cases}OH\\ O\end{cases} + H + H.$$

The nascent hydrogen so formed reduces the reducible substance, methylene blue. Plainly the action of the enzyme is to activate water. Hence the assumption of the presence of H_4O is unnecessary, and the fact that it has never been isolated renders its existence very problematical.

The oxidation of salicylic aldehyde by the juice of the potato in the presence of potassium chlorate is the work of an aldehydase, salicylic aldehyde being a substance

difficult to oxidise. Formic aldehyde and benzyl alcohol are more easily oxidised, and for them the presence of a nitrate is sufficient. That plant and animal tissues have the power of reducing nitrates to nitrites has long been known, and in the light of these facts appears to be a special case of the power of certain enzymes to transfer oxygen (probably as OH) from a substance rich in oxygen to one that is poorer.

Summary.

(1) The processes of oxidation and reduction are not merely opposite, they are also complementary, one implying the other.

(2) Oxidation of saturated substances, either in the laboratory or in the living organism, usually consists in the replacement of hydrogen atoms by hydroxyl groups.

(3) The source of the hydroxyl groups can in some cases be traced to the hydroxyl group of the molecule of water.

(4) There is no evidence that ozone takes any part in biological oxidations.

(5) Owing to the inactivity of molecular oxygen as an oxidiser, its oxygen must be activated before it can be utilised.

(6) In a coupled reaction the oxidation of an easily oxidisable substance brings about the simultaneous oxidation of a difficultly oxidisable substance associated with it.

(7) Three main theories have been formulated to account for the known facts of oxidation: (1) Van't Hoff's ionisation theory, (2) Hoppe Seyler's nascent hydrogen theory, (3) Traube's peroxide theory.

(8) There is evidence that oxidations take place at

times which can be explained in terms of any one of these theories, but the third or peroxide theory explains all the known facts and is open to fewest objections.

(9) The peroxide theory postulates the combination of molecular oxygen with an autoxidisable substance to form a higher oxide, which is immediately transformed into a lower oxide and a peroxide, and the peroxide so formed acts as the oxygen carrier to the substance to be oxidised.

(10) That a peroxide is concerned in biological oxidations is proved by the cases in which hydrogen peroxide is the only known substance which can imitate in the laboratory certain biological oxidations.

(11) It has been thought that hydrogen peroxide is the peroxide concerned, but it can be proved by direct experiment in certain cases that it is not the peroxide formed, and if an organic peroxide is formed there is no necessity to assume that hydrogen peroxide is formed from it before oxidation can take place.

(12) Even on the peroxide theory of oxidation it is probable that the process of oxidation in saturated compounds consists in the replacement of H by OH, the latter being directly derived from the peroxide. In unsaturated compounds oxidation consists in the addition of OH derived from the same sources.

(13) To account fully for the results of oxidation in some cases a subsequent rearrangement of the atoms of the molecule must be assumed.

(14) As the peroxide does not appear to be reformed from the same material, one of the essential qualities of catalytic action appears to be lacking, viz. unlimited action; this appears to be the case with the oxidases. There is evidence, nevertheless, that an enzyme is concerned.

(15) The best known oxidases are enumerated with their main actions.

(16) The cardinal experiments proving that an oxidase is compounded of a peroxide and a peroxidase are described. Peroxidase has all the properties of a true enzyme.

(17) The specificity of the oxidases is not so great as that of the hydrolytic enzymes.

(18) The oxidases are specially sensitive to the action of certain electrolytes.

(19) Analogies with the co-enzymes of the hydrolytic enzymes are found in the action of the hydroxides of manganese, iron, copper, and possibly zinc.

(20) Peroxidase is the most widely distributed of the enzymes. Its activity appears to be one of the most characteristic and persistent properties of living material.

(21) Catalase is an important enzyme with a unique action on hydrogen peroxide, but as it does not activate oxygen it is not an oxidase.

(22) While some doubt has been thrown on the existence of reducases certain experiments given seem to prove their existence.

(23) Analogies with the action of the reducases are to be found in the action of the platinum group of catalysts.

(24) Bach has suggested the existence of a perhydride of oxygen, H_4O, in water, and an enzyme acting on it which he names perhydridase, to account for the formation of nascent hydrogen, as it is this which carries out the reduction.

(25) Aldehydase as an oxidising enzyme derives its oxygen from water.

(26) The origin of the nascent hydrogen required for reduction can be traced to the activation of water without the necessity of assuming the existence of such a substance as H_4O.

(27) The reduction of nitrates by plant and animal tissues appears to be a case of the transfer of oxygen from a substance rich in oxygen to one that is poorer.

The Method of Enzyme Action. A Hypothesis.

If the conclusions derived from the study of hydrolysis and oxidation in the preceding sections are admitted, it follows that hydrolysis and its converse, synthesis, are due to the addition or removal of the H and OH groups, and also that in saturated compounds oxidation is a replacement of H by OH and reduction a replacement of OH by H, while in unsaturated compounds oxidation is an addition of OH and reduction an addition of H. The influence of free oxygen is an indirect one as an acceptor for the hydrogen set free. In other words, the whole of enzyme action has been reduced to the action of hydrogen and hydroxyl radicles.

That this should be so is really to be expected if one thinks of life and living tissue as a development in the presence of the two universal substances, water and air. The processes of hydrolysis and dehydration, oxidation, and reduction can take place without the intervention of catalysts, and it may be that the earliest forms of life depended on the naturally occurring reactions. As, however, life developed and increased in complexity an increase in the speed of the reactions would become necessary, and at the same time a limitation of their sphere of action, for a reaction which at one place would be beneficial at another might be injurious. Two powers are, therefore, required:

(1) Control of the rate of reaction.

(2) Control of the sphere of reaction.

The control of the rate of reaction resolves itself into a

means of controlling the amount and the movements of the H and OH radicles wherever found. The presence of a substance which had an attraction for these radicles and yet did not hold them so firmly as to be unable to give them up if a substance presented itself with greater attractions in the given circumstances, would solve the problem of increasing the rate of reaction, since now the reaction would be due not to the naturally occurring, sparsely distributed radicles, but to masses of them. A general increase, however, of these radicles would act on all parts of the vital structure equally; this danger must be avoided by control of the sphere of reaction. This can be attained by limiting the masses of radicles to definite points to which the substances to be acted on are attracted, and not other bodies present in the solution. The limitation to definite points is secured by the colloid nature of the enzymes, as the action takes place not generally in the solution but only on the surfaces of their particles. The further limitation that only certain substances of those present are to be acted on is ensured by attracting only these substances to the active points. The attraction may be either physical or chemical, but as the physical in the shape of adsorption appears to precede the chemical action in all cases, we may assume that the enzyme forms an adsorption compound with the substrate always. By these means complete control of the reaction is obtained, its speed is increased, and its action limited to the desired point and the desired substances.

Any enzyme, therefore, possesses two qualities.

(1) A power common to it and all other enzymes of attracting one or both of the H and OH radicles.

(2) A specific power of adsorbing a particular substrate or substrates.

These powers, the specific and the general or unspecific,

are to be found in all enzymes, but the degree of the power varies; one enzyme may be weaker than another in its attraction for H or OH, and the specificity of a particular enzyme may on the one hand be limited to a particular substrate and on the other may extend to a large group of substances.

While the specific attractions of enzymes indicate extreme diversity of composition, the possession of a power common to all enzymes indicates the action of a limited class of substances. These two apparently contradictory inferences are only reconcilable by assuming that the majority of enzymes are compounded of two substances, one exercising the specific and the other the general function. A small class of enzymes may exist single in composition because that member of the limited class which can exercise the general function happens also to attract a particular substrate. In general, however, the double action must indicate a double composition.

While it seems almost impossible to arrive at the composition of the specifically acting member of the alliance it ought not to be impossible to discover the class of substances which can exercise the general function, as this class is likely to be limited, and members of it should be discoverable by a study of those enzymes about whose composition most is known.

In spite of the complication of the entire process which takes place in oxidation, when the actual enzyme action takes place the process appears to be among the simplest of these actions, as it has been reduced to peroxide-peroxidase-substrate, and there are other indications that in oxidation enzyme action is to be found in its simplest form. In the first place the extreme specificity of some of the hydrolytic enzymes is not met with among the oxidases as a rule, laccase oxidising not merely laccol but

many aromatic hydroxy-derivatives as well; secondly, laccase at any rate is not so sensitive to heat as are the hydrolytic enzymes, for its solutions can be boiled for three minutes without destroying their activity. While this enzyme is especially stable to heat, other oxidases are destroyed only at 80–90° C., though the maximal temperature for the hydrolytic enzymes varies from 60° to 75° C. Lastly, the extraordinarily wide distribution of peroxidase or the peroxidases, for they are practically universal in living cells, indicates a simple constitution, since otherwise it is difficult to understand how it or similar substances could be found in cells of such diverse nature.

That the oxidising enzymes are the simplest also appears from the fact that most is known of their action and of the bodies which influence them. It has already been mentioned that the salts of manganese and iron seem to be intimately connected with oxidation, and in a lesser degree copper is also active, and possibly zinc, and, further, that the active preparation is the hydroxide. Manganese and iron, even apart from their relation to the oxidising enzymes, can greatly influence the process of oxidation, *e. g.* manganese salts will bring about the oxidation of hydroquinone in air. If a trace of ferrous sulphate (less than one part per million) be added to old tincture of guaiacum or to a fresh tincture of guaiacum containing a trace of hydrogen peroxide, a very intense blue coloration is obtained. On heating the solution containing ferrous sulphate and hydrogen peroxide it loses its power to blue guaiacum, and traces of mineral acids also prevent this action just as they prevent the action of peroxidases, the dosage being the same in both cases. Again, Wolff claims to have reproduced all the actions of the oxidases with colloidal ferrocyanide of iron. It acts as peroxidase towards phenols: it is filterable without loss of activity;

its activity is weakened after one minute's boiling, and traces of mineral acids reduce its activity; it is also sensitive to excess of hydrogen peroxide. Sjolleman has made a colloidal solution of manganese oxide which gives the characteristic reactions of the oxidases and decomposes hydrogen peroxide like potassium permanganate.

The resemblance of manganese and iron hydroxide action to the oxidases is increased when in the presence of albumin and similar colloidal substances. In dilute solution of fresh egg albumin made faintly alkaline a small amount of manganese chloride will give the guaiacum reaction, and heat destroys the oxidising power of this like other similar compounds. Dony Henault obtained a very active preparation by precipitating a solution containing 10 grams of gum arabic, 1 gram of manganese formate, and 0·4 gram of crystallised sodium carbonate in 50 c.c. of water with alcohol; this was filtered off, washed, reprecipitated, and redissolved. In the end it resembled natural laccase very closely. In all the cases mentioned the solutions were slightly alkaline, and the OH ions, if not absolutely necessary, certainly greatly stimulate the process.

The hydroxides of manganese and iron are substances insoluble in water; they can be obtained in the colloidal form, but being suspensoid colloids they are easily precipitated. Suspensoid colloids can have conferred on them the properties of emulsoid colloids in this respect if a certain amount of emulsoid colloid is added to the suspensoid, and it is believed that adsorption between the two takes place. Hence the action of albumin, gum arabic, etc., may be a stabilising action on the manganese. If such a compound is formed it ought to show the other properties of the emulsoid from which it is derived, such as sensitiveness to heat and to alcohol, and since the

number of emulsoids from which to choose is practically unlimited, the specificity of the combination would also be due to the emulsoid. Bayliss concludes, therefore, that a peroxidase is in all probability a peculiarly active form of the colloidal hydroxide of manganese, iron, or copper, preserved in this active state by the presence of an emulsoid colloid, and that it is to this stable colloid that the enzyme owes its precipitation by heat and alcohol, and possibly any degree of specificity that it may possess. He states that a view essentially the same as this was suggested by Perrin.

If manganese and iron hydroxides are the active molecules they must possess some property relatively to oxygen which is peculiar to them, and not shared by the hydroxides of other metals, or by very few of them. That there is such a property is shown by the fact that there are at least two simple hydroxides of manganese, and the same is true of iron. When manganese chloride, $MnCl_2$, is treated with an alkaline hydroxide *in the absence of air* a colourless flocculent precipitate of manganous hydroxide, $Mn(OH)_2$, is formed. Exposed to air the white precipitate rapidly absorbs oxygen and passes into manganic hydroxide, $Mn(OH)_3$. Again, white ferrous hydroxide is precipitated when an alkaline hydroxide or ammonia is added to a solution of a ferrous salt, *provided air be entirely absent.* Exposed to air the white precipitate rapidly absorbs oxygen and passes into ferric hydroxide, $Fe(OH)_3$. Therefore for both manganese and iron there are two hydroxides possible, the higher differing from the lower by the possession of an extra OH, and also by being the more stable form. That, however, $Fe(OH)_2$ can exist as well as $Fe(OH)_3$ while there is no indication of the existence of FeOH, proves that the third OH of the hydroxide $Fe(OH)_3$ is not so firmly bound to Fe as the other two. This agrees with

the fact that reducing substances can reduce ferric salts to ferrous. It follows, therefore, that $Fe(OH)_2$ is such a substance as appeared to be demanded in an enzyme, viz. one that had an attraction for either H or OH, in this case OH, yet did not hold it so firmly as to be unable to give it up in the presence of a substance with greater attractions in the given circumstances. But if a substance exist which can take up OH and give it out again as required, then that substance would at the end of the reaction appear unchanged, and in this respect would conform to the definition of a catalyst.

In an oxidase reaction, therefore, $Fe(OH)_2$ or $Mn(OH)_2$ ought to be able to take the place of peroxidase, and that this is possible is proved by the facts already given, which show the immense stimulating effect of ferrous and manganous salts. The mechanism of the reaction may be conceived to be as follows. Three substances are present—a peroxide, R—O—OH, manganous hydroxide, $Mn(OH)_2$, and a substance to be oxidised, or $Fe(OH)_2$ might be used instead. Assuming that the substance to be oxidised is a saturated compound, its oxidation will consist in the replacement of H by OH, and it must, therefore, contain replaceable H. It may be represented as H.Sub. So long as it is in this form the OH of the peroxide, though, as has been pointed out, but weakly attached, cannot detach the H from H.Sub, therefore R—O—OH + H.Sub produce no reaction. But what is impossible for weakly attached OH is quite possible for the strongly negative radicle R—O— if it be brought into existence by the detachment of OH. This is effected by the attraction of $Mn(OH)_2$ for OH, which is stronger than the weak attraction of R—O— for it. Hence the reaction is:

$$R—O—OH + Mn(OH)_2 = RO^- + Mn(OH)_3$$

The strongly negative radicle RO^- has a great attraction for positive H and detaches it from H.Sub.

$$RO^- + H.Sub = ROH + Sub\pm.$$

It follows from this that RO is much more strongly negative than Sub; in fact, in most oxidations *in vivo* Sub must be practically amphoteric in its reactions, being negative to strongly positive radicles like H and positive to strongly negative radicles like OH. It is, therefore, represented as Sub±. In virtue of its positive character to OH and the fact that it cannot exist uncombined it must have a stronger attraction for OH than has $Mn(OH)_2$, which is able to exist as such. Therefore the last stage of the reaction is:

$$Sub^+ + Mn(OH)_3 = Sub.OH + Mn(OH)_2,$$

and as a result the substrate is oxidised and the catalyst reformed.

This appears to be satisfactory for saturated substances, but not for unsaturated where simple addition of OH takes place, and no H is present to unite with the residue of the peroxide. In such cases the strongly negative RO appears to be able to derive the necessary hydrogen from water.

$$R.O.OH + Mn(OH)_2 = RO^- + Mn(OH)_3$$
$$RO^- + HOH = ROH + OH^-$$
$$2Sub + Mn(OH)_3 + OH^- = 2Sub.OH + Mn(OH)_2.$$

In this case an OH radicle is freed in addition to the OH which is attached to $Mn(OH)_2$, and consequently two molecules of the unsaturated substance can be oxidised, one deriving its OH from $Mn(OH)_3$ and the other from the OH radicle freed in the reaction. Probably the action of a catalyst would be rarely required, however, with an unsaturated substance which as a strongly reducing body can take its OH direct from the peroxide.

$$\dot{R}.O.OH + Sub = RO^- + Sub.OH$$
$$RO^- + HOH = ROH + OH^-$$
$$OH^- + Sub = Sub.OH.$$

Since water must be dissociated in this reaction two molecules of the substrate are oxidised.

The whole explanation is based on the assumption of intermediate compounds with the catalyst, which later break up with reformation of the catalyst. That such intermediate compounds are formed in all cases is not certain, but that they are found in some appears to be clear from the action of MnO_2 in the decomposition of potassium chlorate described in the section on catalysis, and it has been proved by Brode in one case, that of the acceleration of the reaction between hydriodic acid and hydrogen peroxide by molybdic acid. A series of permolybdic acids is formed by the action of hydrogen peroxide on the catalyst; these are formed with great rapidity, and when formed they react with great rapidity also on hydriodic acid with separation of iodine and return of the catalyst to its original form of molybdic acid.

As regards enzymes, the existence of intermediate adsorption compounds appears to be beyond doubt, but whether the enzyme enters into the chemical reaction is unknown. A further examination of the reactions given, however, seems to throw some light on the point.

There seem to be two weak points in the equations: (1) The absence of the action of water in the case of saturated substances; (2) the splitting of undissociated molecules of water by RO^-. As regards the first, if water enters into the reaction in the case of an unsaturated substance it is hardly likely to be inactive in the case of a saturated. Taking in the action of water the first set of equations becomes:

$$R.O.OH + Mn(OH)_2 = RO^- + Mn(OH)_3.$$
$$RO + HOH = ROH + OH^-.$$
$$H.Sub + OH^- = Sub.OH + H^+.$$
$$H^+ + Mn(OH)_3 = Mn(OH)_2 + HOH.$$

It has already been pointed out that the radicle Sub must have but small attraction for H, and if the above be the correct form of the equations it must have less for it than for OH. This is likely, as it is a substance capable of being oxidised, and the oxidised form is usually more stable than the unoxidised. The hydrogen radicle set free reforms the catalyst by uniting with its feebly combined OH.

With regard to the second point—the dissociation of HOH—it is true that RO is strongly negative, but it is hardly likely to be so strongly negative as OH, and there does not seem any good reason why it should attract H away from it. It is also true that H and OH ions occur in water, but they are so small in amount that it is difficult to believe that they have any appreciable effect on the reaction.

But while it is unlikely that RO^- could detach H from OH in a free molecule of water it might be able to do this provided the attachment of OH to H were weakened in any way. Now, $Mn(OH)_2$ has a considerable attraction for OH, and though this is not nearly sufficient to break its connection with H (unless an acceptor for the separated H is present like dissolved oxygen) it might weaken the connection if its attraction for OH were sufficient to attach a molecule of water to itself, thus :

$$Mn(OH)_2 - - OH'H.$$

In such a compound the attraction of OH for H would not be so great as in the free water molecule, and the radicle RO^- might be able to break the connection, particularly as this does not involve the appearance of a free

OH radicle. The final form of the equations then would be:

$$R.O.OH + Mn(OH)_2 - - OH'H = ROH + Mn(OH)_3 + OH^-$$

$$H.Sub + OH^- = Sub.OH + H^+.$$

$$H^+ + Mn(OH)_3 = Mn(OH)_2 - - OH'H.$$

The reformation of the catalyst in this conception certainly means the extraction of OH from its combination with $Mn(OH)_2$ but not its complete severance, and the real catalyst is not $Mn(OH)_2$ but the hydrated form $Mn(OH)_2 - - OH'H$. Such a body would not be a chemical compound proper, it would be a molecular compound, yet one depending on chemical affinity.

In the case of an unsaturated compound the only difference would be that the OH'H molecule is not reformed by the course of the reaction, but is derived from a fresh molecule of water.

At the commencement of this section the presence of a substance capable of massing the H and OH radicles by its attraction for them, yet not holding them so firmly as to be unable to give them up, was postulated. If $Mn(OH)_2$ can really form such a compound as $Mn(OH)_2 - - OH'H$, then that compound has the qualities required.

An objection may be raised to the equations given on the ground that R.O.OH would react with $Mn(OH)_2 - - OH'H$ as follows:

$$R.O.OH + Mn(OH)_2 - - OH'H = ROH + Mn(OH)_4,$$

$$Mn(OH)_4 = HOH + MnO(OH)_2 \text{ (manganous acid)},$$

and that there would then be no oxidation of the substrate and no reformation of the catalyst. It does actually happen, if a solution containing a peroxide and a peroxidase is allowed to stand without any substrate, that the solution loses its enzymic activity, and the reason is now clear. $Mn(OH)_4$ is a very unstable compound and rapidly loses water, hence there is no great tendency to

its formation, and a substrate, if present, takes up OH in preference.

Granting that such a compound as $Mn(OH)_2 - - OH'H$ can exist, a compound formed on the opposite model attracting H and not OH ought to exist, X - - H'OH. It would not be a peroxidase, for the semi-detached radicle now is OH, not H. If this OH were taken up by a substance containing H, which had an attraction for OH and the H were replaced, nascent hydrogen would be set free and this could perform reductions. This appears to occur with the platinum group of metals, and especially with palladium.

$R.CHO + Pd - - H'OH = R.COOH + Pd.H + H^+$.

H^+ + oxidised indigo = reduced indigo + OH^-.

$Pd.H + OH^- = Pd - - H'OH$.

Hence reducing enzymes may be of the type X - - H'OH. It has been recognised that in these reactions another substance is oxidised, and that the necessary oxygen is derived from water. Both these facts are satisfied by the above equations.

If the existence of $Mn(OH)_2 - - OH'H$ be granted as a chemical molecular compound, then in a number of such compounds the strength of the attraction for OH, or, in the case of X - - H'OH, for H must vary, some attracting the radicle but weakly and others strongly. It the attraction be very strong the second water radicle must be very readily formed, and the catalyst be a powerful one. Hence palladium is a more powerful catalyst for certain reactions than platinum, for while platinum black can occlude one hundred times its volume of hydrogen, palladium black can occlude six times as much. It is unlikely that any chemical-atomic compound is formed with the hydrogen, and if not, such cases are of great interest, as they indicate that if a physical attraction for one com-

ponent of a chemical compound is powerful enough, that compound can be broken up provided an acceptor for its other component is present.

With regard to the specific element of the oxidases there is little information to be had except for one striking fact. There seems to be a difference between the laccase derived from the Rhus genus of plants and that from the Medicago genus. The former always contain manganese, the latter, though their activity is greatly intensified by manganese compounds, do not contain it, nor do they contain iron, etc., which might take its place. Further, they are much less sensitive to heat, for while the former are destroyed by boiling for a short time the latter are unaffected. On chemical examination the latter are found to be composed of a mixture of calcium salts of organic mono- and polybasic hydroxyacids, among which are glycollic, $CH_2OH.COOH$, malic, $COOH.CHOH.CH_2.COOH$, mesoxalic, $COOH.C(OH)_2.COOH$, and citric acids. When a mixture of the pure salts is made, its catalytic power is found to be practically that of Medicago oxidase. From a consideration of the chemical formulæ it is clear that, apart from their combination with calcium, their point of agreement is the possession of alcoholic hydroxyl. It may be that there is a tendency to further oxidation in the group containing this, and that this tendency leads to the formation of molecular compounds with water. However this may be, the catalyst so formed is to a certain extent specific in that it acts on the group of substances attacked by laccase, and it might be possible by examination of the physical and chemical qualities of the salts of these hydroxyacids to say wherein the attraction resides.

Medicago laccase, therefore, does not conform to the type of double composition which the facts seem to

require in the majority of enzymes. It is representative of that small class, where the substance exercising the general function also possess a specific attraction for the substrate.

It is now possible, so far as the oxidases are concerned, granting the existence of the compound with water, to answer the question whether enzymes take part in the chemical reaction which they accelerate. The answer is that they do *not* directly. Their function is confined to enabling the hydrogen radicle obtained from molecules of water to carry out the reaction. A similar position is occupied by the reducing enzymes, the only difference being that these activate the hydroxyl radicle of water. In neither case does the enzyme take part directly in the reaction; its function is indirect and consists in activating one or other of the water-radicles.

The composition of the hydrolysing enzymes is not so well known as that of the oxidases. There is nothing to compare with the constancy of manganese or iron in the latter. It is true that they are colloidal, and that they have a double action, specific in relation to their substrates, but general in their action on those substrates. This, however, does not prove that they are necessarily double in composition, or even if they are, that the unspecific component is a comparatively simple compound. Still, analogy with the oxidases is in favour of a similar composition, and the fact that simple bases and acids can carry out hydrolyses in proportion to their ionisability is greatly in favour of the simplicity of the unspecific component, for ionisation is not met with to the same extent in the more complex organic bases and acids.

The existence of co-enzymes is another indication of

the simplicity of the unspecifically acting component of a hydrolytic enzyme. In many cases none are known, in others they are known to exist but their composition is unknown, in still others they are familiar chemical substances. If an unspecific component exists, it might be thought that it should be capable of detection in all cases, but this leaves out of account the very variable character of the firmness of attachment in adsorption compounds. Fixation often occurs, *e. g.* trypsin to charcoal, so that the compound cannot be broken by mere washing with water, and yet it can hardly be described as chemical, since another substance more strongly adsorbed can release it. We may expect to find some co-enzymes very loosely attached, others more firmly, while again we may suspect the existence of others which we cannot detach. As examples, it may be mentioned that pancreatic lipase can be separated from its co-enzyme by mere filtration of the glycerol extract. In this case the co-enzyme may be a bile-salt. Sodium chloride, the co-enzyme of liver amylase, is more firmly attached, for it can be removed only by dialysis. The co-enzyme of yeast juice requires for its separation high pressure on a gelatinised filter. Where none can be found by these methods, the ash may give evidence of the presence of a more or less simple co-enzyme, but not necessarily, for if it be an organic compound or an ammonium salt it would not leave traces in the ash. It may be asserted that co-enzymes are only adjuvants of enzymes, but in face of the fact that the enzyme action in many cases cannot be carried on without them, they are more likely to be essential parts of the enzymes.

That mineral acids and bases can catalyse hydrolyses is well known, but the method of action is not known. It has been pointed out previously that the existence of

either a free OH or a free H ion would account for the hydrolysis. Now, since a base, *e. g.* KOH, ionises into K and OH, and an acid, *e. g.* HCl, into H and Cl, it follows that the ion required is in either case present, so that the action may be on the following lines, XY being the substance to be hydrolysed:

$$K^+ + OH^- + XY = X^- + YOH + K^+$$
$$K^+ + H^+ + OH^- = KOH + H^+$$
$$H^+ + X^- = HX$$

For an acid:

$$H^+ + Cl^- + XY = HX + Y^+ + Cl^-$$
$$Cl^- + H^+ + OH^- = HCl + OH^-$$
$$Y^+ + OH^- = YOH.$$

There are, however, difficulties in accepting these equations as a true picture of the reactions. The difficulty does not lie in the decomposition of XY, for in such a substance as ethyl acetate, which is a typical substance hydrolysed by hydrochloric acid, the acetic residue represents a weak acid only, not to be compared with hydrochloric. The difficulty is in explaining why a double decomposition of the ordinary type does not take place, $HCl + XY = HX + YCl$. In part the explanation may be the small attraction of Y, *i. e.* ethyl, for Cl, since the formation of ethyl chloride only takes place in a mixture of hydrochloric acid and ethyl alcohol when both alcohol and acid are as nearly pure as possible. There remains the difficulty of explaining why the H radicle should leave HCl to attach itself to the acetic radicle when acetic is a so much weaker acid than hydrochloric. The objection appears to be fatal to any attempt to represent the facts on these lines. If, however, it were got over, the further reaction with water is explained by the small attraction of Y for Cl and the presence of the naturally occurring ions of water. When these are removed by combination with

Y and Cl fresh ions are formed to restore the equilibrium which exists between the undissociated water molecules and the water ions. These criticisms and explanations apply also to hydrolysis by KOH. Another explanation must, therefore, be sought for.

The process would be represented on Werner's scheme as follows: HCl is not the true active acid but an anhydro-acid which has a strong attraction for the OH of HOH through its H component; hence it hydrates, OH entering the first or non-dissociable zone, and H the second or dissociable zone, thus:

$$HCl + HOH = (ClH.OH)H$$

so forming the true or aquo-acid. This dissociates:

$$(ClH.OH)H = (ClH.OH)^- + H^+.$$

With a substance XY a double decomposition occurs:

$$(ClH.OH)^- + H^+ + XY = (ClH.OH)Y + HX.$$

The fate of (ClH.OH)Y depends on the strength of the attraction of ClH for OH. The whole ionises into $(ClH.OH)^- + Y^+$. It may go further by hydrolysis:

$$(ClH.OH)^- + Y^+ = ClH + OH \quad + Y^+.$$

Being a hydrolysis this is a reversible equation, Y appearing on both sides of the equation is without effect. Therefore by the law of mass action the product of the concentrations of ClH and OH divided by the concentration of (ClH.OH) is a constant. If OH increases ClH.OH must increase, *i. e.* in alkaline solution there will be practically no hydrolysis. But if OH diminishes ClH must increase to keep the product constant. Now the solution is not alkaline but acid owing to the presence of HX, therefore hydrolysis occurs:

$$(ClH.OH)Y = ClH + YOH.$$

The anhydro-acid is reformed and hydrolysis of XY has taken place.

Similarly with bases, KOH is the anhydro-base which

has a strong attraction for the H of HOH through the OH component, and there is formed the aquo- or true base (KHO.H)OH. Double decomposition occurs with XY, forming (KHO.H)X + YOH, the latter ionising as Y^+ and OH^-. As the solution is therefore alkaline, hydrolysis of (KHO.H)X takes place with the formation of HX and the reformation of KOH.

It is possible to use the notation already explained when describing the action of the oxidases, the apostrophe ' being used as before to indicate weakened bonds.

$$KOH + HOH = KHO--H'OH$$
$$KHO--H'OH + XY = KHO--H'X + YOH$$
$$KHO--H'X = KOH + HX.$$

With the necessary changes the same equations apply to the action of HCl.

There is not, however, the same reason for the molecular union with water as there was in the case of $Mn(OH)_2$, and if it occurs it must be put down to some such cause as the auxiliary valency which Werner postulates. The arguments for the union of water with acids, bases, and salts have been given earlier and are very cogent. Werner states that $HCl(OH_2)_2$ has actually been proved to exist.

An objection may be raised on the ground that in acid hydrolysis (ClH.OH)Y (or ClH--OH'Y, according to the notation employed) is supposed to be formed, and in the case of the hydrolysis of methyl acetate by HCl this would be methyl chloride. Now Tafel has pointed out (1) that methyl chloride is not formed with any rapidity under the conditions of the reaction, and (2) it cannot replace HCl as a catalyst. The answer to the first objection is that the hydrated methyl chloride supposed to be formed appears to have only a momentary existence, as it must be unstable in the presence of excess of H ions; also that it is a hydrated methyl chloride, and it is doubtful

that methyl chloride added in the anhydrous state would hydrate so readily in solution as HCl. The answer to the second point is that if a catalyst acts by forming intermediate compounds, methyl chloride cannot act as a catalyst unless such an unlikely body as methyl chloride of methyl were formed.

It follows from the views propounded in this discussion of the action of acids and bases that the H and OH radicles required are derived from the water of the solvent, just as occurs with the oxidases.

While acids and alkalies are efficient hydrolysers they are not to be compared with the enzymes as regards power. Boiling with hydrochloric acid for hours is needed to produce the same change in proteins that trypsin can produce in an hour or two at body temperature, and the great power of lactase on milk sugar in comparison with a mineral acid has already been mentioned. On this account it might be thought that enzyme action was essentially different from the H and OH action of acids and bases, were a simpler explanation not available. The enzymes concentrate the radicles at the point of action and also the substrate, viz. the surfaces of their particles, whereas in acid or alkali hydrolysis both substrate and ions are evenly dispersed through the solution; hence much fewer collisions between ions and substrate molecules take place with the latter in a given time.

The hydrolytic action of salts, which such facts as the necessity of sodium chloride for liver amylase and bile-salts for pancreatic lipase oblige us to assume, is more difficult to account for. Of the four possible classes of salts, (*a*) those of a strong acid and a strong base, (*b*) of strong acid and weak base, (*c*) of weak acid and strong base, and (*d*) of weak acid and weak base, there is no difficulty in seeing how H ions can be formed in (*b*) and

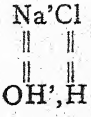
OH ions in (*c*), for these salts hydrolyse to some extent in aqueous solution, liberating a strong acid from class (*b*) and a strong base from class (*c*). These give rise to H or OH ions in the manner described in the preceding section. As regards class (*d*) hydrolysis, it is true, is much more marked than in the other two classes, but there is little ionisation and, presumably, little attraction for water, hence few H or OH ions are formed. Class (*a*), however, is of special interest because practically no hydrolysis takes place in aqueous solution, yet the facts compel us to assume that H or OH radicles arise if the hypothesis of H or OH action in all cases is correct. Such facts are the activation of liver amylase by sodium chloride, and the activation of trypsin by calcium chloride.

While these salts of a strong acid and a strong base are not hydrolysable by simple solution in water, there is evidence that in certain circumstances they do hydrolyse. Emich's experiment with red-hot sodium chloride and water in the spheroidal state shows the influence of temperature; but more important in the present connection is the acidity of the supernatant fluid when a suspensoid colloid is precipitated by barium chloride. It was shown in a previous section that this could be accounted for satisfactorily only on the hypothesis that hydrolysis of the barium chloride had occurred. Barium as an alkaline earth forms a strong base, and, therefore, barium chloride falls into the class of salts under consideration. Now the compound formed by the colloid and barium is clearly an adsorption compound; if, therefore, adsorptive attraction be strong enough it can induce a chemical action between two other substances which would not react without it—in this case barium chloride and water. The action seems to be similar to that of palladium in oxidising a hypophosphite in solution; the hypo-

phosphite under its influence reacts with water. The action has been traced already to the combination of palladium with the hydrogen of water, and this combination, considering how it is affected by physical agents, is not likely to be a chemical one, though it may be due to solid solution not adsorption.

It is true that monovalent radicles like sodium will not precipitate an emulsoid colloid except in comparatively high concentration, but nevertheless when present in lesser concentration the tendency must exist, *i.e.* the hydrolytic action of the colloid on the salt must be in its first stage. Since there is evidence that salts in solution unite with water, the combination with water is not the first stage of the hydrolysis, for the combination takes place whether the colloid is present or not. The first stage then must be a weakening of the bond between the atoms composing the salt: NaCl becomes Na'Cl. The combination with water only can be represented in Werner's form as (NaHO.H)Cl, or in the notation used in this section as:

If the bond between the Na and Cl is weakened by the colloid the attraction of Na for OH, on the one hand, and of Cl for H on the other, will be strengthened, and this will be accompanied by a corresponding weakening of the bond between OH and H, thus:



In presence of a substrate, XY, with which either NaOH or HCl can react, I imagine there would be a

momentary snapping of the bond between Na and Cl, with the result that two reactions would take place:

$$Na\,OH + XY = NaX + YOH$$

and

$$HCl + XY = YCl + HX.$$

Of the four bodies so formed two would react as follows:

$$NaX + YCl = NaCl + XY,$$

and there would be left NaCl reformed, XY, substrate, still attracted to the enzyme, while HX and YOH, the products, would diffuse away, the reaction recommencing as before.

This is the simplest form of the reaction. It is probable that at the moment of formation of NaQH and HCl other molecules of water enter the reaction, preventing the formation of NaX and YCl and conforming the reaction to the equations given previously for hydrolysis by an acid and an alkali.

So far the explanations have dealt with hydrolysis; it remains to show how synthesis may occur. Synthetic reactions occur when water is in defect, while yet the attraction of the catalyst for either H or OH remains as before. Unable to obtain the required radicle from water it finds it in one of the bodies to be united, thus:

$$HCl + YOH = ClH - - OH'Y.$$

Double decomposition then occurs with HX owing to the attraction of OH for H above its attraction for Y:

$$ClH - - OH'Y + HX = ClH - - OH'H + XY.$$

An equilibrium exists between the concentration of the substances on the enzyme and that of those in the solution which this increase of water on the enzyme has disturbed, therefore the water attached to the catalyst must be detached and diffuse away.

$$ClH - - OH'H = HCl + HOH.$$

Equilibrium will be reached when the concentration of water in the solution has so increased that there is no tendency for water formed by the above reaction to diffuse away; the catalyst is then hydrated and cannot act on HX and YOH.

Similarly for an alkali catalyost:

$KOH + HX = KHO--H'X$

$KHO--H'X + YOH = KHO--H'OH + XY$

$KHO--H'OH = KOH + HOH.$

For a salt of the NaCl type, where owing to its colloid connection NaCl has become Na'Cl, the reactions may be thus represented:

$Na'Cl + YOH + HX = Y'OH--Na'Cl--H'X$

$Y'OH--Na'Cl--H'X = NaOH + X^-$ and $HCL + Y^+$

$= NaX + YCl + HOH$

$NaX + YCl = NaCl + XY.$

Hence for synthesis as well as hydrolysis the efficient agents are the H and OH radicles.

So far the unspecific element of a hydrolytic enzyme has been dealt with. As regards the specific element little seems to be known. A hint may possibly be found in the fact mentioned already: "An addition of 0·05 grm. of asparagine to 100 c.c. of a starch solution containing amylase increases the velocity of the reaction sevenfold." Asparagine is the monamide of aspartic acid and is perhaps the most common amide in plants. Its chemical solution is $HOOC.CH_2.CH(NH_2).CONH_2$, and it exists in optically isomeric forms. It is a weak acid, and as such may have some slight effect in increasing the hydrogen ion concentration, but it ought to be far surpassed in this particular by stronger acids. It follows, therefore, that its extraordinary influence cannot be due to this, and that it is more probably due to its action on

the specific component of the enzyme. If this specific component were asparagine, or were in part composed of asparagine, then the addition of asparagine would be equivalent to adding fresh enzyme, for the unspecific component is probably present in the solution ready to combine with it. A study of the physical and chemical qualities of asparagine and its action on starch—whether, for example, it forms adsorption compounds with starch with peculiar facility—ought to throw light on the constitution of amylase.

SUMMARY.

(1) The whole of the chemical action of enzymes may be reduced to the action of hydrogen and hydroxyl.

(2) This is to be expected, as the two substances everywhere present when life first developed were water and air.

(3) The use of enzymes is to control the rate and the sphere of naturally occurring reactions. The former is secured by increasing the number of the active H and OH radicles; the latter by limiting them to definite points to which the substrate of the reaction is also attracted.

(4) Any enzyme possesses a general or unspecific power of attracting one or other of the H and OH radicles, and a specific power of adsorbing a particular substrate.

(5) This double power probably indicates a double constitution.

(6) The enzyme action which takes place in oxidation appears to be one of the simplest forms.

(7) The hydroxides of manganese and iron are intimately connected with peroxidase action. They can by themselves influence the process of oxidation, and their resemblance to the enzymes is greatly increased in the presence of colloids.

(8) A peroxidase is probably a hydroxide of manganese, iron, etc., preserved in the colloidal form by an emulsoid colloid. It is to the latter component that the reactions of the enzyme to heat and alcohol and its specificity are due.

(9) The hydroxides of manganese and iron are found in two forms, differing from each other in stability and in the possession or not of an extra OH radicle.

(10) Equations are given showing the reactions that a hydroxide of manganese in the presence of a peroxide and a substrate would be likely to initiate, in both saturated and unsaturated substances.

(11) The action depends on the formation of intermediate compounds; the existence of these is proved in some cases of catalytic action.

(12) On close examination the equations given are unsatisfactory unless the entrance of water into the reaction is assumed. It is shown also that the water so entering must be in a form in which the bond between its H and OH radicles is weakened.

(13) It follows that the hydroxide of manganese as such is not capable of carrying out the functions of peroxidase; it must first be hydrated. The act of hydration loosens the bonds connecting H and OH in the water combined.

(14) Since substances such as the lower hydroxides of manganese and iron exist which have the power of attracting OH, it is likely that substances can be found which have a similar power of attracting H. Such substances would act as reducing catalysts.

(15) The action of palladium and platinum black is explained on this hypothesis.

(16) The specific element in the oxidases is unknown, but some of the laccases give evidence bearing on its constitution.

(17) Enzymes do not take part directly in the chemical action which occurs; their part is the activation of one or other of the water-radicles, and it is this latter which carries out the reaction.

(18) That the hydrolytic enzymes have a similar double composition may be conjectured from analogy with the oxidases, and also from the existence of co-enzymes. The latter appear in many cases to be essential to the action of the enzyme.

(19) The catalytic action of the mineral acids and bases in hydrolysis is discussed, and it is shown that the facts are difficult to explain except on the hypothesis of hydration of the acid or base, resulting in the weakening of the bond between the H and OH radicles in the combined water.

(20) Objections raised in the particular case of the hydrolysis of methyl acetate in the presence of hydrochloric acid, based on the improbability of methyl chloride being an intermediate compound, are considered and possible answers given.

(21) The more powerful action of enzymes, as compared with acids and bases, is traced to concentration of the H and OH radicles.

(22) The hydrolytic action of salts in connection with enzymes is discussed. It is shown that of the four classes of salts, two form acids and bases by hydrolysis, and another is probably not concerned with enzyme action.

(23) The large class of salts formed from a strong base and a strong acid raises the greatest difficulty, but it is shown that in special circumstances they can hydrolyse into acids and bases, and evidence is given for the probability that union with a colloid leads to the first step towards this hydrolysis, viz. a weakening of the bond which holds their component radicles together.

(24) Union between the salt and water takes place apart from the presence of a colloid, and this union weakens the bond between the H and OH radicles of the combined water. When the union with the colloid has weakened the bond between the components of the salt, the bond connecting the H and OH of the combined water is still further weakened.

(25) In presence of a substrate which can be acted on, both acid and alkali may be formed simultaneously from the salt, and these, reacting with the substrate and with each other, cause hydrolysis of the substrate and reformation of the salt.

(26) It is shown that synthetic reactions can be accounted for on the same basis of union of the catalyst with H and OH, but in synthetic reactions this union takes place with the H and OH radicles of the two substrates, not with those of water.

(27) The specific elements of the hydrolytic enzymes are unknown, but facts bearing on the constitution of this component of amylase are mentioned.

Deductions.

The hypothesis of enzyme action to which this inquiry has led is based on (1) the possibility of combination between molecules, and (2) the hypothesis that this molecular combination loosens the internal bonds in one or both of the combining molecules. As regards the possibility of combination between molecules the existence of the double salts appears to put this beyond doubt, and for the existence of combinations of solute and solvent in the case of water arguments have already been given. To these may be added the fact that when crystals of almost any salt are dissolved in water a lowering of the

temperature takes place owing to the development of osmotic pressure. The development of this pressure implies that work has been done, and for this work energy must be obtained from somewhere. It is obtained by extracting heat from surrounding objects. If, on the other hand, the *anhydrous* salts are dissolved, there is often a rise of temperature which must be due to combination with the solvent setting free an amount of heat which is not merely sufficient to supply all the energy required for the work done in developing osmotic pressure, but which is able also to supply heat to surrounding objects. With regard to the second point—the loosening of internal bonds—this may furnish an explanation of chemical action between non-electrolytes where ions do not appear to be formed, for the loosening of the internal bonds must render the substances affected more reactive, in fact it may be an essential condition of reaction. One is tempted to suggest that molecular combination precedes chemical action in all cases, and is the analogue in the molecular world of the adsorption which precedes chemical action in the case of colloids.

Natura non facit saltum is an aphorism which is applicable. There ought to be all stages between that where two bodies can remain in intimate juxtaposition without any kind of reaction and the stage where the two react with explosive violence, and one of these stages may be that of molecular union with loosened atomic bonds. Such a conception implies that a valency on an atom is not an individual unitary force pointed in a definite direction, but a certain quantity of affinity which is divisible and may be altered in position. This view is held by Werner. It is usual to speak of residual affinity as accounting for molecular compounds, but there does not seem any necessity to assume the existence of this in

order to explain these compounds. The proof of the existence of unsatisfied affinities in certain cases depends on quite other considerations. If the affinity of an atom is concentrated on one part of its surface (if surface can be said to exist on an atom) directly opposite to a similarly affected surface on another atom with which it is combined, it is conceivable that the entrance of a compound on the scene containing an atom with which the first could combine if both were free, may cause a certain amount of the affinity on both to leave its previous position and be concentrated on those sides of the atoms which face. If the change in distribution of the affinity is sufficient, the combination on both sides will break up and double decomposition occur; but if not, a certain attraction will still be exercised, resulting in molecular combination. As the affinity of the atom is to some extent redistributed, there is less attracting that atom with which it is already combined, and this implies a loosening of this bond. The loosening of the bond implies that a certain amount of affinity on the other combined atom is unsatisfied, and this is indicated by an increase in its reactivity. In the most stable compounds, no doubt, the amount of affinity which is subtracted from that uniting the partners is insufficient to weaken the union materially. The affinity is so great that there is, so to say, plenty to spare.

Any hypothesis of enzyme action must account for the properties of enzymes. Most of those described in the section dealing with these properties have already been accounted for, *e. g.* specificity, synthetic or reverse action, combination with substrates and products, catalytic action, and co-enzymes. Of the remainder, the influence of electrolytes which are not co-enzymes may be mentioned. The very great influence of acids and alkalies can now be easily accounted for by their production in

the solution of H or OH ions. Since the whole enzymic process, so far as it is chemical, depends on the H and OH radicles, the addition of hydrogen or hydroxyl ions derived from an outside source must have a profound influence. If the action is due to the hydrogen radicles, the addition of a small amount of hydrogen ions may assist the process, but if they are added in excess the production of the radicles by the enzyme may be interfered with and even suppressed. Since the rapidity and power of the enzymic reaction depends on the local concentration of these radicles, a suppression of this concentration by acid will reduce the process to one of ordinary acid hydrolysis, which is much slower than enzyme hydrolysis. The same remarks apply to the addition of alkali to a solution containing an enzyme acting by the production of OH radicles. If, on the other hand, hydrogen ions are added to an enzyme acting by OH radicles, or hydroxyl ions to one acting by H radicles, then a neutralisation of the enzymic radicle will take place and the enzyme action disappear.

The action of salts is more complicated. If they do not combine with the unspecific component of the enzyme and yet are adsorbed by its colloid they may aid the reaction, but if they combine they may destroy the enzyme action altogether. They may also combine with the substrate, as indeed may acids and alkalies, and the combination may be more readily adsorbed by the enzyme, or adsorption may be prevented altogether. However they act, the fact that they must practically always have some action on one or other of the components of the enzyme reaction is amply sufficient cause for their well-known great influence on enzymes.

The great influence of heat may be accounted for in several ways. Chemical reactions are accelerated by

increasing the temperature; hence, in moderation a rise of temperature may increase the speed of an enzymic reaction, but an immoderate increase destroys the enzyme. It has been pointed out that colloids are sensitive to heat, in many of them irreversible coagulation occurring if the temperature rises too high. This appears to be due to aggregation of their particles and probably also to a change in the composition of these particles. As enzymes have a colloid component a rise of temperature beyond a certain point must affect this component and therefore the whole enzyme. Coagulation would remove the unspecifically acting component in a state of adsorption. There is, however, probably another cause at work. If molecular combination precedes chemical action, it may be that the adsorption of the general component to the specific is due to the possibility of chemical combination taking place between them in other circumstances. The rise of temperature may render this chemical combination possible, and then the enzyme ceases to be an enzyme. A condition of this kind is seen in the combination of the blue acid of Congo-red with a colloidal hydroxide of aluminium, iron, etc. The adsorption compound is blue, the colour of the free acid, but if it be heated to 100° C., combination takes place and the red colour of the salt appears.

The existence of anti-enzymes is disputed, but they may exist if the colloid component of the enzyme can act as an antigen, *i. e.* if it be a protein, as it may be in some cases. An antibody appears to unite with its antigen, for the neutralisation of antigen by antibody is quantitative. The combination so formed may cease to attract the generally acting component or the substrate, and the enzyme as enzyme cease to exist. On the other hand, merely altering the hydrogen ion concentration must have

an enormous effect on any enzyme, so that it must be difficult to decide which cause is at work.

Zymogens may be accounted for in certain cases where an acid or alkali can act as activator by a chemical decomposition setting free the enzyme from an inactive combination, but it is also possible that the zymogen is the colloidal component of the enzyme without its generally acting component, and that the action of the activator is to supply this.

The ultimate proof of an hypothesis is the demonstration of the truth of deductions made from it. If the hypothesis I am urging is a correct explanation of enzyme action it ought to be possible to manufacture enzymes. Clearly defined principles from which the constitution of the enzyme required by a particular substrate could be theoretically deduced are still wanting, but it is possible to devise an empirical method for arriving at the same result. It is necessary first of all to discover a colloid with special attractions for the substrate under consideration, and tests for the presence of this might be based on the following points. It is clear that a sufficient aggregation of molecules of substrate about a colloid particle will increase its size, so that a tendency to turbidity if not precipitation may occur. The disturbing effect of electrolytes in altering the electric charge must be eliminated. This increase in the size of the colloid particles might be seen in the ultramicroscope, or may be macroscopically visible as an increased turbidity; or again it might be proved by precipitation of the combination by an electrolyte which was previously unable to effect this. An example of the last is furnished by mixing dialysed amylase and starch, which produces no precipitation; if to the mixture sodium chloride is added precipitation

occurs, though sodium chloride was unable to precipitate either component separately.

The unspecific component of the required enzyme may be found by adding various salts, acids, or alkalies to the solution containing the specific colloid. While it is not necessary that the salt, acid, or alkali to be found should be inseparable from the colloid, it certainly should be difficult of removal, this indicating the formation of an adsorption compound. The number of salts, acids, and alkalies utilisable is not innumerable. Apart from the oxidation processes, the electrolytes of living tissues are practically summed up in four acids and five bases and their combinations. These are: hydrochloric, sulphuric, phosphoric, and carbonic acids, and the bases of sodium, potassium, calcium, magnesium, and ammonium. Some idea of the strengths in which these substances should be used may be obtained from the analysis of the ash of the purest known enzymes. As all unite with water it is probable that more than one would serve as the non-specific component, though probably one would be more efficient than the others. It might be objected that the efficient hydrolytic agent to be sought for would be an organic compound, but the facts learnt from the co-enzymes leave little doubt that, as a rule, the common electrolytes are the active agents. By proceeding on somewhat similar lines Dony Henault and others have been successful in making artificial oxidases; artificial hydrolytic enzymes ought also to be possible.

The composition and method of action of enzymes may seem a limited subject to expend energy on, but its importance is really considerable. The insight an accurate knowledge of the subject (which is not yet attainable) would give to a physician into the metabolism of special

tissues might lead to true views as to the cause of any fault in this metabolism, and might enable him to rectify it by comparatively simple methods such as the injection of a simple electrolyte or non-electrolyte into the general circulation or into the part affected. The cytologist would find a knowledge of the enzymes of a cell and their action of still greater interest, as the major part of cell chemistry is based on them. He would have at his disposal the means of influencing the functions of a particular cell by supplying it by, for example, Barber's method of puncture, with a slight excess beyond normal of a particular electrolyte, for the enzyme formed in connection with that particular electrolyte would be increased in quantity and its action in the cell increased to an equivalent degree. It would be more difficult to influence the specific component of the enzyme, as this, being of a complex nature, is composed of large molecules or aggregations of molecules. Nevertheless even this component might be influenced if fractions of it were supplied, *e. g.* in the case of the proteins, the amino-acids or the products of their decomposition. These on reception into the cell might be built up into new colloid particles, which in their turn might form particular enzymes. Finally, some of the most characteristic properties of living tissue might be conferred on synthetic colloids, for the combination of these with the appropriate non-specific component might mean the formation of substances which would no longer be, so to say, the passive subjects of reactions, but would be active agents in attacking other substances. So one step more would be made on the road to the great goal of the biologist, the synthesis of life itself.

INDEX.

Acceptor, 86
Acetaldehyde, 66
Acids, strengths of, 69
Acids and alkalies, action on enzymes, 47, 119–123, 133
— — effect on colloids, 17
Acid salts, 51
Activators of enzymes, 48, 52
Actor, 86
Adsorbed material, condition of, 27
Adsorption, 22
— causes of, 27
— and chemical attraction, 29
— as a cause of hydrolysis, 78, 124
— compounds, 22, 23
— — definiteness, 24
— distinguished from solution, 23
— effect of heat on, 26
— and electrolytes, 28
— and enzymes, 41, 49, 50
— and fixation, 25, 129
— formula, 25
— rapidity of process of, 26
— reversibility of, 25
— and surface tension, 28
Aldehydase, 101
Amino-acids, hydrolysis of, 63
Amphoterous electrolytes, 18
Amylase, 9, 41, 46, 49, 50, 51, 54, 127, 128, 136
Anaerobic fermentation, oxidation in, 101
Anti-emulsion, 54
Anti-enzymes, 54, 135
Antiseptics, effect on enzymes, 8, 44
Antitryptic serum, 55
Armstrong's view of synthetic action, 39
Asparagine and enzyme action, 54, 127
Atomic bonds, loosening of, 132–133
Autolysis, 9
Autolytic enzymes, 47
Auxiliary valencies, 75

Bases, strengths of, 69
Bile-salts as co-enzymes, 53, 119
Brownian movement, 13

Calcium salts, action on enzymes, 49, 52, 56
Cane-sugar, inversion of, 62
Carbohydrates, hydrolysis of, 62
Catalase, 50, 51, 94, 98
Catalysis, 1
— of oxygen production from potassium chlorate, 2
Catalytic action, resemblances to enzyme action, 42
— — differences from enzyme action, 44
Charcoal as adsorbent, 22
Chymosin, 64
Co-enzymes, 52, 118
Cold, no effect on enzymes, 46
Collodion membranes and enzymes, 51
Colloidal complexes, 20

Colloids, 9
— and chemical constitution, 20
— chemical reactions in, 19
— and crystalloids, 10
— effect of electrolytes on, 14, 16
— action of neutral salts on, 18
— osmotic pressure of, 13
— precipitation or coagulation of, 16
— and surface tension, 11
— viscosity of, 14
Colloid solutions, causes of permanency of, 12
Combination of enzyme and substrate, 39
— solute and solvent, 72, 131
Combustion, intermediate compounds in, 84
Condensation of carbon dioxide on charcoal, 4
Consecutive reactions, 3
Continuous phase, 10
Copper and oxidation, 98
Coupled reactions, 86

Dialysis, 10, 49, 52
Diastase, 47
Diffraction of light by colloid particles, 11
Disperse phase, 10
Double salts in solution, 74

Ecto- and endo-enzymes, 8
Ecto-enzymes, preparation of, 8
Electric charge on colloid particles, 12
Electrolytes, effects of, on colloids, 14 16
— — on enzyme action, 44, 47, 123–126, 133
— effect of passage of current through solutions of, 68
Electrolytic dissociation, 69
Emich's experiment, 71, 124
Emulsion, 35, 37, 40, 41, 47
Emulsoid colloids, 14, 15
Endo-enzymes, preparation of, 9
Enterokinase, 56
Enzyme action, effect of concentration, 43
— methods of investigation, 35
— the method of, 105
— power of, compared with inorganic catalytic action, 34, 123
— specificity of, 35
Enzymes as catalysts, 52
— classification of, by action, 61
— definition of, 8
— effect of heterogeneity of system, 42
— function of, on chemical reactions, 118
— general considerations on, 7
— irreversible changes in, 51
— action of, in low concentrations, 40
— manufacture of, 136
— properties of, 34, 133
— stability of, influenced by acids and alkalies, 48
— two qualities of, 106
— universal occurrence in organism, 7
Equilibrium position under enzyme action, 44
— in solution, 5
Erepsin, 38, 51
Esterases, 62
Ethyl acetate, hydrolysis of, 5, 62, 120
External phase, 10

False equilibrium, 43
Fats, hydrolysis of, 62
Fibrin ferment, 64
Fermentation, 65

Gases, solubility of, in solutions, 72
Gelatinisation, 15, 16
Gels, 15, 16
Gibbs theorem, 27
Glucose, hydrolysis of, 65
Glucosides, action on isomeric, 35
Glyceric aldehyde, 65
Guaiacum blue reaction, 95

Hæmocyanin, 98
Hæmoglobin, 94
Hæmolysis and lipase, 54
Hardy's rule, 17
Heat coagulation, 46
Heat, influence on enzymes, 45, 134
Hofmeister series, 18
Hoppe Seyler's theory of oxidation, 86
Hydration of acids, 77
— of salts, 76
Hydrochloric acid as catalyst, 5
— — and hydrolysis of ethyl acetate, 62
— — and pepsin, 56
Hydrocyanic acid and catalase, 50, 97
Hydrogen peroxide and catalase, 50
— — catalysis of decomposition of, 2
— — in oxidation, 87
— — oxidation by, compared with biological oxidations, 88
Hydrolysis, 61
— through adsorption, 78, 124
— effect of concentration of hydrogen ions on, 79
— of fats, 62
— general equation, 63
— of protein, 63
— Arrhenius' theory of, 70, 72
— by water alone, 42
— Werner's theory of, 71
Hydrolytic enzymes, 118
— — specific elements of, 127
Hydroquinone, 35
Hydroxides of iron and manganese, 110
Hydroxynitriles, decomposition of, 64

Imbibition, 17
— effect of acids and alkalies on, 17
Indigo, oxidation of, 88, 91
Indophenol oxidase, 94
Inductor, 86
Intermediate compounds in oxidation processes, 113
Intermediate compounds in catalysis, 3
Internal phase, 10
Inversion of cane-sugar, 62
Invertase, 34, 40, 47, 51, 52
Ions, 67
— combination of, with solvent, 74
— of gas, 86
Iron hydroxides, 110
— and oxidation, 98, 108–111
— salts, effect on pepsin, 52
— and tyrosinase, 97
Irreversible changes in enzymes, 51

Laccase, 45, 92, 94, 95, 96, 97, 107, 108, 117
Lactase, 34, 37, 47, 123
Lactic acid, 66
Liesegang's phenomenon, 20
Light absorption by solutions, 73
— effect on enzymes, 46
Lipase, 36, 41, 47, 48, 49, 52, 53, 54, 62, 119
Lyophile emulsions, 14

Maltase, 35, 37
Manganese dioxide as catalyst, 2, 3
— hydroxides, 110
— in laccase, 97
— and oxidation, 97, 108–116
"Masked" salts, 27
Methyl chloride as intermediate product, 4, 122
Methylene blue, reduction of, 99
Microscopic vision, limitation of, 11
Minute amount of catalyst, 2
Molecular compounds, 76, 131, 133
Molybdic acid and oxidation, 2, 113

Narcotics, 51
Neutral salt action on colloids, 18
Nitriles, hydrolysis of 65
Nuclease, 47

Optical isomers, action on, 36

Optimum temperature, 46
Organised and unorganised ferments, 8
Ortho-esters, relation to hydration, 65
Osmotic pressure, 13, 132
— — and electrolytic dissociation, 74
— — of solutions of electrolytes, 68
Ostwald's generalisation of Gibbs' theorem, 27
Oxidase action not truly enzymic, 93
Oxidases, 45, 94, 96, 108
— specific elements of, 117
Oxidation, 84
— in anaerobic fermentation, 101
— by enzymes, 107
— Hoppe Seylers' theory of, 86
— and hydroxyl, 90
— of indigo, 88, 91
— and iron, 98, 108–111
— and manganese, 97, 108–116
— of the polyphenols, 92
— of saturated compounds, 84
— Traube's theory of, 87, 88
— of unsaturated compounds, 91
— Van't Hoff's theory of, 86
Ozone, 85

Palladium as catalyst, 100, 116, 125
Papain, 55
Pepsin, 47, 52
Pepsinogen, 48, 56
Perchloride of mercury and enzymes, 51
Perhydridase, 100
Perhydride of oxygen, 100, 101
Peroxidase, 47, 96, 98, 108, 110
Peroxides in oxidation, 87, 89
Phases, homogeneous and heterogeneous, 9
Phosphates and enzymes, 50, 53, 54
Platinum as catalyst, 1, 2, 99
Poisoning of enzymes, 51
Polymerisation of complex molecules, 20
Polyphenols, oxidation of, 92
Potassium chlorate, decomposition of, 2
— permanganate, action on lipase, 52
Products, effects on enzymes, 40, 45
Proenzymes, 47
Proteins as colloids, 18
— hydrolysis of, 63
— Ionisation of, 18
Ptyalin, 49, 54
Purin oxidases, 94
Pyrocatechol, 35
Pyruvic acid, 66
— aldehyde, 66

Radium rays and enzymes, 46
Reducases, 99, 116
Reducing bodies and oxidases, 97
Reduction, 84, 99
Rennet, 64
Resorcinol, 35
Reverse action, 38, 126
Reversible equations, 5
Retarding catalysts, 1
Röntgen rays and enzymes, 46

Salts, action on enzymes, 49, 123–126, 134
Silicic acid, 15
Sodium chloride and enzymes, 50, 54, 119, 136
Sols, 15
Specific adsorption, 30
— surface, 11
Specificity among catalysts, 6
— of enzyme action, 35
— of non-enzymic actions, 35
Stalagmometer, 28
Substrate, 36
— effect of concentration of, 43
Sulphur dioxide, reaction catalysed,
Surface film, compression in, 27
— — solubility in, 27
— tension, 27
Suspensoid colloids, 14
Synthesis of maltose 38

Synthetic action, 38, 126

TEMPERATURE, effect on electrolytic resistance of dilute solutions, 74
— — on enzymes, 45
— — on hydrolysis by water, 81
Toluene not injurious to enzymes, 44
Traube's theory of oxidation, 87, 88
Trypsin, 45, 51, 55, 123
Trypsinogen, 49, 56
Tyndall-Faraday phenomenon, 10
Tyrosinase, 94, 96, 97

ULTRAMICROSCOPE, 10
Ultraviolet rays and enzymes, 36
Urea, hydrolysis of, 63
Urease, 51

VALENCY, definition of a, 132
— of ions, effect on colloids, 16
Valency, Werner's theory of, 75
Van't Hoff's theory of oxidation, 86
Velocity of action of enzymes and catalysts, 43, 44
— — factors of, 43, 48
Viscosity of colloids, 14

WATER and combustion, 85
— electrolytic dissociation of, 69
— as a hydrolyser, 70, 80
— molecular combination of, 76
— necessary for synthetic action, 38
— and oxidation, 85, 113
Werner's theory of valency, 75, 132

YEAST juice, 8, 53, 119

ZINC and oxidation, 98
Zymase, 50, 51, 53, 54
Zymogens, 47, 49, 52, 55, 136

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